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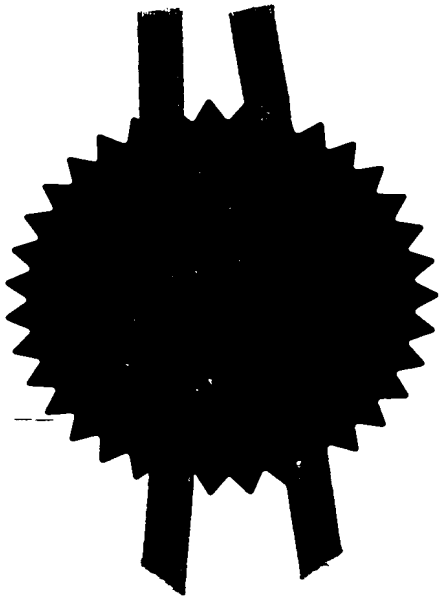

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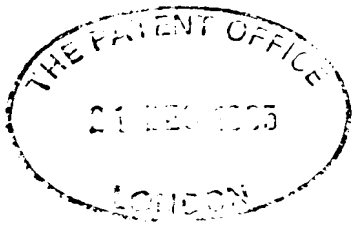

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F31/7700 25.00

Your reference PA387

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Form 1/77

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1 Please give the title of the invention: RECOMBINANT CHIMERIC RECEPTORS

❷ Applicant's details

❑ **First or only applicant**

2a If you are applying as a corporate body please give:

Corporate name: CELLTECH THERAPEUTICS LTD

Country *(and State of incorporation, if appropriate)*:

2b If you are applying as an individual or one of a partnership please give in full:

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Name DR. A.E. LOVE

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Postcode SL1 4EN

Daytime telephone number *(if available)* 01753 534655 ext 2375

ADP number *(if known)* 637352b001

❹ Reference number

4 Agent's or applicant's reference number *(if applicable)* PA 387

❺ Claiming an earlier application date

5 Are you claiming that this application be treated as having been filed on the date of filing of an earlier application?

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❽ *Please supply duplicates of claim(s), abstract, description and drawing(s).*

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Continuation sheets for this Patents Form 1/77			
Claim(s)	/	Description	25
Abstract	/	Drawing(s)	32

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RECOMBINANT CHIMERIC RECEPTORS

This invention relates to an improved method of activating a cell, a DNA delivery system, a DNA sequence coding for a recombinant chimeric receptor, target cells and target hosts containing said DNA delivery system, to a method of treatment comprising administering said DNA delivery system; to the use of said DNA delivery system in medicine.

The natural T-cell receptor is a complex association of polypeptide chains comprising antigen binding, transmembrane and cytoplasmic components. Binding of antigen to the receptor in the correct context triggers a series of intracellular events leading to activation of the T-cell and for example destruction of the antigen presenting target cell. Before recognition of the antigen can take place, the antigen must be presented in association with MHC molecules. It would be highly desirable if this requirement for MHC in presentation of an antigen could be bypassed and T-cells engineered to become active on binding ligands other than a natural MHC-presented antigen. This would provide a means of avoiding the variability between individuals associated with MHC presentation and would also permit the targeting of more highly expressed surface antigens thereby increasing the efficacy of lymphocyte mediated therapy e.g. tumour therapy.

Chimeric receptors have been designed to target T-cells to cells expressing antigen on their cell surface. Such recombinant chimeric receptors include chimeras containing binding domains from antibodies and intracellular signalling domains from the T-cell receptor, termed 'T-bodies', see for example Published International Patent Applications Nos. WO 92/10591, WO 92/15322, WO 93/19163 and WO 95/02686. The recombinant chimeric receptors described in the art are composed of a ligand binding component, a transmembrane component and a cytoplasmic component. It has been found however, that transfection of T-cells with these recombinant chimeric receptors does not result in acceptable levels of T-cell activation upon antigen binding unless the T-cell is also co-stimulated by, for example, treatment with high levels of Il-2. Such treatment using T-cells transfected with the recombinant chimeric

receptor makes the method suitable principally for *ex-vivo* treatment of patients. Treatment of patients *ex-vivo* is a lengthy and complicated procedure.

The present invention offers an alternative to the present *ex-vivo* approach and achieves improved *ex-vivo* activation without the need for addition of costimulating agents such as Il-2, and successful *in-vivo* redirection and activation of T-cells bearing a recombinant chimeric receptor. The invention further provides a means of increasing cell activation in response to a single type of extracellular interaction. As used herein, cell activation may be evidenced by an increase in proliferation; expression of cytokines with, for example pro or anti-inflammatory responses; stimulation of cytolytic activity, differentiation or other effector functions; antibody secretion; phagocytosis; tumour infiltration and/or increased adhesion.

The invention provides an effector cell with two or more different signalling cytoplasmic components which are not naturally linked and which advantageiously are chosen to act together cooperatively to produce improved activation of the cell. This may be achieved using a DNA delivery system comprising one or more DNA sequences coding for a recombinant chimeric receptor comprising two or more signalling cytoplasmic components which are not naturally linked and where at least one of said cytoplasmic signalling components is derived from a membrane spanning polypeptide. Alternatively the DNA delivery system may comprise two or more recombinant chimeric receptors each comprising one or more different signalling cytoplasmic components which are not naturally linked and where at least one of the cytoplasmic signalling components is derived from a membrane spanning polypeptide. DNA coding for such recombinant chimeric receptors may be introduced into T-cells or other effector cells *in-vivo* and/or *ex-vivo*. Subsequent binding of an effector cell expressing one or more chimeric receptors to a target cell elicits signal transduction leading to activation of the effector cell in a process involving clustering or dimerisation of chimeric receptors or allosteric changes in the chimeric receptor or another mechanism for receptor-triggering.

In a first aspect the invention provides a method of activating a cell as a result of one type of extracellular interaction between said first cell and a cell surface target molecule on a second cell characterised in that said first cell is provided with a DNA delivery system comprising one or more DNA molecules coding for two or more different cytoplasmic signalling components which are not naturally linked, and wherein at least one of said cytoplasmic components is derived from a membrane spanning polypeptide.

When the DNA coding for the signalling cytoplasmic components is expressed, and on the extracellular interaction between the cell and a cell surface target molecule on a second cell, a signal is transduced via the cytoplasmic components to two or more different intracellular signalling messengers resulting in activation of the cell.

The signalling cytoplasmic components may form part of a recombinant chimeric receptor and the cell is transfected with a DNA delivery system comprising DNA coding for a recombinant chimeric receptor where the receptor comprises two or more different signalling cytoplasmic components which are not naturally linked and wherein at least one of the signalling cytoplasmic components is derived from a membrane spanning polypeptide. The recombinant chimeric receptor is expressed on the cell surface and on binding of a cell surface target molecule two or more intracellular responses are produced via the signalling cytoplasmic components.

The recombinant chimeric receptor preferably comprises a binding component capable of recognising a cell surface molecule on a target cell, and a transmembrane component in combination with the signalling cytoplasmic domains.

In a second aspect the invention provides a DNA delivery system comprising one or more DNA molecules coding for a recombinant chimeric receptor in association with a carrier said receptor comprising two or more different signalling cytoplasmic components which are not naturally linked,

and wherein at least one of said cytoplasmic components is derived from a membrane spanning polypeptide.

As used herein the term 'not naturally linked' is used to denote signalling cytoplasmic components which in nature are not connected to each other on a single polypeptide chain.

The DNA may comprise two or more DNA molecules which together form one recombinant chimeric receptor. For example, each DNA molecule comprises DNA coding for a signal peptide component, a binding component, a transmembrane component and one or more signalling cytoplasmic components. Each DNA molecule may comprise DNA coding for a different number of signalling cytoplasmic components. Upon expression within the target cell and/or target host the resulting polypeptide chains assemble to form a recombinant chimeric receptor.

In a preferred embodiment of the second aspect of the invention, the invention provides a DNA delivery system comprising DNA coding for a recombinant chimeric receptor in association with a carrier wherein said DNA codes for:

i) a signal peptide component

ii) a binding component capable of recognising a cell surface molecule on a target cell

iii) a transmembrane component
and

iv) two or more different signalling cytoplasmic components and wherein said cytoplasmic components are not naturally linked, and wherein at least one of said cytoplasmic components is derived from a membrane spanning polypeptide.

The DNA delivery system may also comprise DNA coding for two recombinant chimeric receptors each with one or more signalling

cytoplasmic components where one or more of the components is derived from a membrane spanning polypeptide.

In a third aspect the invention provides a DNA delivery system comprising two or more DNA molecules coding for two or more recombinant chimeric receptors wherein each of said receptors comprises one or more different signalling cytoplasmic components and said different signalling cytoplasmic components are not naturally linked, and wherein at least one of said cytoplasmic components is derived from a membrane spanning polypeptide.

The recombinant chimeric receptors of the third aspect of the invention preferably also comprise a binding component, a transmembrane component and one or more different signalling cytoplasmic components, said different signalling cytoplasmic components not being naturally linked, and the DNA molecule coding for the receptor preferably also comprises DNA coding for a signal peptide component.

The components of the recombinant chimeric receptor are operatively linked such that the signalling cytoplasmic components are functional in transducing a signal resulting in activation of one or more messenger systems as a result of recognition of a cell surface molecule on a target cell by the binding component.

Two or more of the components may be linked by one or more spacer regions. The spacer regions may function to facilitate the components adopting the correct conformation for biological activity. The use of a spacer region to link the transmembrane component and the binding component is particularly advantageous.

The spacer regions may for example comprise up to 300 amino acids and preferably 20 to 100 amino acids and most preferably 25 to 50 amino acids.

Spacers may be derived from all or part of naturally occurring molecules such as from the immunoglobulin like components of CD8, e.g. the CD8

hinge region; CD4; CD28; an antibody constant component, or may be a non-naturally occurring sequence. All or part of natural spacing components between functional parts of intracellular signalling molecules for example spacers between ITAMS (immunoreceptor tyrosine based activation motifs) may also be used.

In a particularly preferred embodiment of the second aspect of the invention there is therefore provided a DNA delivery system comprising DNA coding for a recombinant chimeric receptor in association with a carrier wherein said sequence comprises DNA coding for:

i) a cell signal component

ii) a binding component capable of recognising a cell surface molecule on a target cell

iii) a transmembrane component

iv) two or more different signalling cytoplasmic components, said cytoplasmic components not being naturally linked and wherein at least one of said cytoplasmic components is derived from a membrane spanning polypeptide

and wherein two or more of said components may optionally be linked by one or more spacer regions.

The binding components may be all or part of a molecule interacting with cell surface molecules and may be chosen to recognise a surface marker expressed on cells associated with a disease state such as for example those associated with virally infected cells, bacterially infected cells, cancer cells, such as the bombesin receptor expressed on lung tumour cells; peptide hormones, adhesion molecules, inflammatory cells present in autoimmune disease, or a T-cell receptor or antigen giving rise to autoimmunity.

Suitable binding components for use in the constructs of the invention also include all or part of receptors associated with binding to cell surface

associated molecules; the T-cell receptor; CD4; CD8; CD28; cytokine receptors e.g. an interleukin receptor, TNF receptor, interferon receptor e.g. γ-IFN; receptors for colony stimulating factors e.g. GMCSF; antibodies and antigen binding fragments thereof including for example Fab, Fab', $F(ab')_2$, scFv, Fvs, V_H and V_L components which may be in association with C_H and C_L domains; and where the antibodies or fragments may be murine, human, chimeric or engineered human using techniques well known in the art (see for example International Patent Application WO 91/09967).

Where the DNA delivery system comprises two or more DNA molecules coding for two or more recombinant chimeric receptors, the binding component of each recombinant chimeric receptor participates in the same type of extracellular binding event for example they both bind to the same ligand expressed on the same tumour cell. It is preferred that the binding components bind to the same or different epitopes of the same antigen and it is particularly preferred that the binding component of each recombinant chimeric receptor is the same.

The transmembrane component may or may not be naturally linked to the cytoplasmic component to which it is attached either directly or by means of a spacer. Transmembrane components may be derived from a wide variety of sources such as the zeta chain of the T-cell receptor, CD28, CD8, CD4, cytokine receptors e.g. interleukin receptor, TNF receptors, interferon receptors, colony stimulating factor receptors e.g. GMCSF.

The extracellular spacer and transmembrane components may be chosen such that they have free thiol groups thereby providing the construct with multimerisation capacity, such as for example CD28 components and the zeta chain of the natural T-cell receptor, and antibody hinge sequences.

The signalling cytoplasmic components for example transduce a signal which results in activation of one or more intracellular messenger system. It is preferred that each of the cytoplasmic components activates a different messenger system. Examples of suitable cytoplasmic components include, for example those derived from the T-cell receptor

zeta, eta or epsilon chain; CD28, Fc receptors e.g. the γ chain of FcRI, signalling components from cytokine receptors e.g. interleukin, TNF and interferon receptors, colony stimulating factor receptors e.g. GMCSF; tyrosine kinases e.g. ZAP-70, fyn, lyk, Itk and syk; and signalling components of adhesion molecules e.g. LFA-1 and LFA-2. The signalling cytoplasmic components are preferably ITAM containing cytoplasmic components

The binding component, transmembrane component, and cytoplasmic components are preferably derived from or based on human sequences.

The intracellular messenger systems which may be activated either directly or indirectly include, for example, one or more kinase pathways such as those involving tyrosine kinase, PKC or MAP kinase; G-protein or phospholipase mediated pathways; calcium mediated pathways; and pathways involving synthesis of a cytokine such as an interleukin e.g. IL-2, including NFAT, and cAMP mediated pathways.

The peptide signal component may be that naturally associated with the binding component or may be derived from other sources. Examples of suitable signal peptide components include immunoglobulin signal sequences.

The carrier for use in the DNA delivery systems according to the invention may be a vector or other carrier suitable for introduction of the DNA *ex-vivo* or *in-vivo* into target cells and/or target host cells. Examples of suitable vectors include viral vectors such as retroviruses, adenoviruses, adenoassociated viruses, EBV, and HSV.

The vectors or other carriers may be non-viral vectors which may include promoter/regulatory sequences and/or replication functions from viruses such as retrovirus LTRs, AAV repeats, SV40 and hCMV promoters and/or enhancers, splicing and polyadenylation signals; EBV and BK virus replication functions.

Tissue specific regulatory sequences such as the TCR-α promoter, E-selectin promoter and the CD2 promoter and locus control region may also be used.

Non-viral based vectors such as liposomal vectors and vectors based on DNA compacting agents may also be used.

For *ex-vivo* use, the DNA delivery system of the invention may then be introduced into effector cells removed from the target host using methods well known in the art e.g. transfection, transduction, biolistics, protoplast fusion, calcium phosphate precipitated DNA transformation, electroporation, cationic lipofection, or targeted liposomes.

Where two or more DNA molecules are used in the DNA delivery system they may be incorporated into the same or different carriers as described above.

Examples of suitable effector cells include cells associated with the immune system such as lymphocytes e.g. cytotoxic T-lymphocytes, tumour infiltrating lymphocytes, natural killer cells, neutrophils, basophils or T-helper cells; dendritic cells, B-cells, haemopoetic stem cells, and macrophages. The use of T-lymphocytes is especially preferred.

The effector cells are then reintroduced into the host using standard techniques.

A wide variety of target hosts may be employed according to the present invention such as, for example, mamms and, especially, humans.

The DNA delivery system according to the invention may be in a form suitable for *in vivo* administration. It may, for example, be in the form of a targeted delivery system in which the carrier is capable of directing the DNA to a desired effector cell. Particular examples of such targeted delivery systems include targeted-naked DNA, targeted liposomes encapsulating and/or complexed with the DNA, targeted retroviral systems and protamine and poly-lysine condensed DNA.

Targeting systems are well known in the art and include using, for example, antibodies or fragments thereof against cell surface antigens expressed on target cells *in vivo* such as CD8; CD16; CD4; CD3; selectins e.g. E-selectin; CD5; CD7; CD34; activation antigens e.g. CD69 and IL-2R. Alternatively, other receptor - ligand interactions can be used for targeting e.g. CD4 to target $HIV_{gp}160$ - expressing target cells.

The use of targeted liposomes such as antibody targeted liposomes is preferred.

Particular types of liposomes which may be used include for example pH-sensitive liposomes especially antibody-targeted pH-sensitive liposomes where linkers cleaved at low pH may be used to link the antibody to the liposome.

Cationic liposomes which fuse with the cell membrane and deliver the recombinant chimeric receptor DNA according to the invention directly into the cytoplasm may also be used.

Liposomes for use in the invention may also have hydrophilic groups attached to their surface to increase their circulating half-life such as for example polyethylene glycol polymers. There are many examples in the art of suitable groups for attaching to liposomes or other carriers; see for example International Patent Applications Nos. WO 88/04924, WO 90/09782, WO 91, 05545, WO 91/05546, WO 93/19738, WO 94/20073 and WO 94/22429. The antibody or other targeting molecule may be incorporated in the hydrophilic group as described in the art.

Non-targeted delivery systems may also be used and in these targeted expression of the DNA is advantageous. Targeted expression of the DNA may be achieved for example by using T-cell specific promoter systems such as the zeta promoter and CD2 promoter and locus control region, and the perforin promoter.

In a further aspect the invention provides effector cells transfected with a DNA delivery system according to the invention.

The effector cells according to this aspect of the invention may be any of those previously described above and are preferably T-cells most preferably cytotoxic T-cells.

The DNA delivery system may be a therapeutic or diagnostic composition and may take any suitable form for administration, and, preferably is in a form suitable for parenteral administration e.g. by injection or infusion, for example by bolus injection or continuous infusion. Where the composition is for injection or infusion, it may take the form of a suspension, solution or emulsion in an oily or aqueous vehicle and it may contain formulatory agents such as suspending, preservative, stabilising and/or dispersing agents.

Alternatively, the composition may be in dry form, for reconstitution before use with an appropriate sterile liquid.

If the composition is suitable for oral administration the formulation may contain, in addition to the active ingredient, additives such as: starch - e.g. potato, maize or wheat starch or cellulose - or starch derivatives such as microcrystalline cellulose; silica; various sugars such as lactose; magnesium carbonate and/or calcium phosphate. It is desirable that, if the formulation is for oral administration it will be well tolerated by the patient's digestive system. To this end, it may be desirable to include in the formulation mucus formers and resins. It may also be desirable to improve tolerance by formulating the compositions in a capsule which is insoluble in the gastric juices. It may also be preferable to include the composition in a controlled release formulation.

In a yet further aspect the invention provides the use in medicine of a DNA delivery system comprising one or more DNA molecules coding for one or more recombinant chimeric receptors in association with a carrier said receptor comprising two or more different signalling cytoplasmic components which are not naturally linked.

In a still further aspect of the invention, there is provided a method of treatment of a human or animal subject, the method comprising administering to the subject an effective amount of a DNA delivery system comprising one or more DNA coding for one or more recombinant chimeric receptors in association with a carrier, said receptor comprising two or more different signalling cytoplasmic components which are not naturally linked.

In a further aspect the invention provides DNA molecule coding for a recombinant chimeric receptor wherein said DNA comprises DNA coding for:

i) a signal peptide component

ii) a binding component capable of recognising a cell surface protein on a target cell

iii) a transmembrane component

iv) two or more signalling cytoplasmic components wherein said cytoplasmic components are not naturally linked together as a single translation product.

In a preferred embodiment of this aspect of the invention two or more of said components may optionally be linked by one or more spacer molecules.

Homologues of the individual components of the chimeric receptor may be used. The term homologue as used herein with respect to a particular nucleotide or amino acid sequence coding for a component of the chimeric receptor represents a corresponding sequence in which one or more nucleotides or amino acids have been added, deleted, substituted or otherwise chemically modified provided always that the homologue retains substantially the same function as the particular component of the chimeric receptor. Homologues may be obtained by standard molecular

biology and/or chemistry techniques e.g. by cDNA or gene cloning, or by use of oligonucleotide directed mutagenesis or oligonucleotide directed synthesis techniques or enzymatic cleavage or enzymatic filling in of gapped oligonucleotides.

Fragments of the individual components may also be used wherein one or more nucleotides has been deleted provided that the fragment retains substantially the same function as the starting component of the chimeric receptor.

The DNA for use in this and other aspects of the invention may be obtained from readily available DNA sources using standard molecular biology and/or chemistry procedures, for example by use of oligonucleotide directed mutagenesis or oligonucleotide directed synthesis techniques, enzymatic cleavage or enzymatic filling in of gapped oligonucleotides. Such techniques are described by Maniatis *et al* in Molecular Cloning, Cold Spring Harbor Laboratory, New York 1989, and in particular in the Examples hereinafter.

The DNA delivery system according to the invention may be useful in the treatment of a number of diseases or disorders. Such diseases or disorders may include those described under the general headings of infectious diseases, e.g. HIV infection; inflammatory disease/autoimmunity e.g. rheumatoid arthritis, osteoarthritis, inflammatory bowel disease; cancer; allergic/atopic diseases e.g. asthma, eczema; congenital e.g. cystic fibrosis, sickle cell anaemia; dermatologic, e.g. psoriasis; neurologic, e.g. multiple sclerosis; transplants e.g. organ transplant rejection, graft-versus-host disease; metabolic/idiopathic disease e.g. diabetes.

The invention is further illustrated in the following non-limiting Examples and Figures in which:

Figure 1 shows: diagrammatic representation of recombinant chimeric receptor constructs cloned into pBluescript KS+

Figure 2 shows: diagrammatic representation of recombinant chimeric receptor constructs cloned into pBluescript KS+

Figure 3 shows: oligonucleotide sequences for recombinant chimeric receptor construction

Figure 4 shows: nucleotide and amino acid sequence of an hCTMO1/CD8/zeta recombinant chimeric receptor

Figure 5 shows: nucleotide and amino acid sequence of an hCTMO1/CD8/zeta-CD28 recombinant chimeric receptor fusion

Figure 6 shows: nucleotide and amino acid sequence of an hCTMO1/CD8/CD28 recombinant chimeric receptor

Figure 7 shows: nucleotide and amino acid sequence of an CTMO1/G1/zeta recombinant chimeric receptor

Figure 8 shows: nucleotide and amino acid sequence of an hCTMO1/G1/zeta-CD28 recombinant chimeric receptor fusion

Figure 9 shows: nucleotide and amino acid sequence of an hCTMO1/h/CD28 recombinant chimeric receptor

Figure 10 shows: histogram representation of IL2 production by cell lines TB3.2, 3.13 and 3.24 when stimulated with an anti-idiotypic antibody alone or in combination with an anti-CD28 antibody

Figure 11 shows: histogram representation of the production of IL2 by cell line TB3.13 when stimulated with antigen expressing tumour cells, shown with and without co-stimulation using an anti-CD28 antibody.

Figure 12 shows: histogram representation of IL-2 production by HGT1.2 and HGT1.4 in response to various stimuli

Figure 13 shows: histogram representation of IL-2 production by HGT2.4 incubated with various combinations of antibodies.

Figure 14 shows: schematic representation of recombinant chimeric receptor constructs.

Figure 15 shows: schematic representation of recombinant chimeric receptor constructs

MATERIALS AND METHODS

INTRODUCTION

scFv / CD8 / Zeta Chimeric Receptor

The scFv / CD8 / Zeta chimeric receptor consists of a single chain Fv linked to an extracellular spacer in the form of part of human CD8 hinge, linked to the extracellular, transmembrane and intracellular components of the human T-cell receptor Zeta chain (TCR).

The single chain Fv consists of the leader sequence and variable component of the light chain of the engineered human antibody linked via a $(Gly_4Ser)_5$ linker to the variable component of the heavy chain of the engineered human antibody. The extracellular spacer consists of residues 98 to 142 of the hinge region of human CD8 (Zamoyska *et al* : Cell 43,153-163, 1985).This is linked to residues 22 to 162 of human TCR Zeta comprising extracellular, transmembrane and intracellular regions (Weissman *et al* : PNAS 85, 9709-9713, 1988).

scFv / CD8 / CD28 Chimeric Receptor

The hCTMO1 CD28 chimeric receptor consists of a single chain Fv linked to an extracellular spacer in the form of part of human CD8 hinge, linked to the transmembrane and intracellular component of human CD28.

The single chain Fv consists of the leader sequence and variable component of the light chain of the engineered human antibody linked via a $(Gly_4Ser)_5$ linker to the variable component of the heavy chain of the engineered human antibody. The extra cellular spacer consists of residues 98 to 142 of the hinge region of human CD8 (Zamoyska *et al* : Cell 43 153-163, 1985). This is linked to residues 132 to 202 of human CD28 comprising the transmembrane and intracellular components (Aruffo & Seed : PNAS 84, 8573-8577).

scFv /CD8 / Zeta-CD28 Fusion Chimeric Receptor

The scFv /CD8 / Zeta-CD28 Fusion chimeric receptor consists of a single chain Fv linked to an extracellular spacer in the form of part of human CD8 hinge, linked to the extracellular, transmembrane and intracellular component of human TCR Zeta fused to the intracellular component of human CD28.

The single chain Fv consists of the leader sequence and variable component of the light chain of the engineered human antibody linked via a $(Gly_4Ser)_5$ linker to the variable component of the heavy chain of the engineered human antibody. The extra cellular spacer consists of residues 98 to 142 of the hinge region of human CD8 (Zamoyska *et al* : Cell, 43,153-163, 1985). This is linked to residues 22 to 162 of human TCR Zeta comprising extracellular, transmembrane and intracellular components (Weissman *et al* : PNAS 85,9709-9713, 1988). This is linked to residues 162 to 202 comprising the intracellular component of human CD28.

scFv / G1 / Zeta Chimeric Receptor

The scFv / G1 / Zeta chimeric receptor consists of a single chain Fv linked to an extracellular spacer comprising human IgG 1 hinge, CH2 and CH3, linked to the transmembrane and intracellular regions of human TCR Zeta.

The single chain Fv consists of the leader sequence and variable component of the light chain of the engineered human antibody linked via a $(Gly_4Ser)_5$ linker to the variable component of the heavy chain of the engineered human antibody. The extracellular spacer consists of residues 234 to 243 of human IgG1 hinge, 244 to 360 of CH2 and 361 to 478 of CH3 (Kabat *et al* Sequences of proteins of immunological interest, 1987). This is linked to residues 22 to 162 of human TCR Zeta comprising extracellular, transmembrane and intracellular regions (Weissman *et al* : PNAS 85 ,9709-9713, 1988).

scFv / G1 / Zeta-CD28 fusion Chimeric Receptor

The scFv / G1 / Zeta chimeric receptor consists of a single chain Fv linked to an extra cellular spacer comprising human IgG 1 hinge, CH2 and CH3, linked to the transmembrane and intracellular regions of human Zeta fused to the intracellular region of human CD28.

The single chain Fv consists of the leader sequence and variable component of the light chain of the engineered human antibody linked via a $(Gly_4Ser)_5$ linker to the variable component of the heavy chain of the engineered human antibody. The extracellular spacer consists of residues

234 to 243 of human IgG1 hinge, 244 to 360 of CH2 and 361 to 478 of CH3 (Kabat *et al* Sequences of proteins of immunological interest, 1987). This is linked to residues 22 to 162 of human TCR Zeta comprising extracellular, transmembrane and intracellular regions (Weissman *et al* : PNAS 85 ,9709-9713, 1988). This is linked to residues 162 to 202 comprising the intracellular component of human CD28.

scFv / h / CD28 Chimeric Receptor

The scFv / h / CD28 chimeric receptor consists of a single chain Fv linked to an extracellular spacer consisting of human IgG1 hinge and part of the extracellular region of human CD28, linked to the transmembrane and intracellular regions of human CD28.

The single chain Fv consists of the leader sequence and variable component of the light chain of the engineered human antibody linked via a $(Gly_4Ser)_5$ linker to the variable component of the heavy chain of the engineered human antibody. The extracellular spacer consists of residues 234 to 243 of human IgG1 hinge and residues 118 to 134 of human CD28. This is linked to residues 135 to 202 of human CD28 comprising the transmembrane and intracellular regions (Aruffo & Seed : PNAS 84, 8573-8577).

These chimeric receptors were constructed for the engineered human antibodies CTMO1, directed against human polymorphic epithelial mucin (PEM).and P67.6, directed against human CD33.

CONSTRUCTION OF CHIMERIC RECEPTORS

Each component of the chimeric receptor constructs was either PCR cloned or PCR assembled by standard techniques (PCR Protocols, Innis et al, 1990, Academic Press inc.) and sub-cloned in a cassette format into pBluescript KS+ (Stratagene), see figure 1 and 2.

1. Single chain Fv cassette

hCTMO1

Leader sequence and hCTMO1 VI was PCR cloned from plasmid pAL 47 (WO 93/06231) with oligos R6490 and R6516 (Oligo sequences are shown in Figure 3). R6490 introduces 5' Not I and Hind III sites and R6516 forms part of the $(Gly4Ser)_5$ linker. hCTMO1 Vh was PCR cloned from plasmid pAL 52 with oligos R6515 (forms part of linker) and R6514 (introduces 3' Spe I site. Leader / VI and Vh fragments were then PCR spliced together and the PCR product was restricted with Not I and Spe I and sub-cloned into pBluescript KS+.

Anti-CD33 Antibody - hP67.6

A hP67.6 single chain Fv was similarly prepared and subcloned into pBluescript KS+.

2. CD8 hinge spacer cassette

The CD8 hinge spacer for hCTMO1 TCR Zeta chimeric receptor and hCTMO1 TCR Zeta-CD28 fusion chimeric receptor (which includes a small part of 5' Zeta) was PCR assembled using overlapping oligos: R6494,R6495,R6496 and R6497. The CD8 hinge spacer for hCTMO1 CD28 chimeric receptor was PCR assembled using overlapping oligos: R6494,R6495,R6496 and R6506. Both PCR products were restricted with Spe I and BamH I and sub-cloned into pBluescript KS+.

3. Human TCR Zeta cassette

Human Zeta transmembrane and intracellular components were PCR cloned from human leukocyte cDNA (Clonetech) with oligos R6488 (introducing a 5' BamH I site) and R6489 (introducing a 3' EcoR I site). PCR product was restricted with BamH I and EcoR I and sub-cloned into pBluescript KS+.

4. Human CD28 cassette

Human CD28 transmembrane and intracellular components were PCR cloned from human leukocyte cDNA (Clonetech) with oligos P3240 (introducing a 5' BamH I site) and P3241 (introducing a 3' EcoR I site). PCR product was restricted with BamH I and EcoR I and sub-cloned into pBluescript KS+.

5. Hinge-CD28 cassette

Human CD28 extracellular, transmembrane and intracellular components were PCR cloned from human leukocyte cDNA (Clonetech) with oligos S0146 (introducing a 5' Spe I site) and P3241 (introducing a 3' EcoR I site). S0146 also constitutes residues 234 to 243 of human IgG1 hinge.

6. Zeta-CD28 fusion cassette

The 3' end of Zeta, starting at a naturally occuring Sty I site and the intracellular component of human CD28 were PCR assembled such that the Zeta stop codon was removed and an inframe fusion protein would be translated. PCR assembly carried out with overlapping oligos: P3301, P3302, P3303, P3304, P3305 and P3306. PCR product was restricted with Sty I and EcoR I and sub-cloned into pBluescript containing the hCTMO1 TCR Zeta chimeric receptor construct, replacing the 3' end of Zeta.

7. Human IgG1 cassette

Human IgG1 hinge, CH2 and CH3 were PCR cloned from IgG1 cDNA clone (A. Popplewell) with oligos S0060 (introducing a 5' Spe I site) and S0061 (introducing a 3' BamH I site. PCR product was restricted with Spe I and BamH I and sub-cloned into pBluescript.

All chimeric receptor constructs were completely sequenced (Applied Biosystems, Taq DyeDeoxy Terminator Cycle Sequencing, Part Number 901497) in pBluescript prior to cloning into the expression vectors.

EXPRESSION OF CHIMERIC RECEPTOR CONSTRUCTS

chimeric receptor constructs were cloned from pBluescript into the expression vectors ee6HCMVNe and ee6HCMVGpt Bebbington (1991), Methods 2, 136-145) on a Hind III to EcoR I restriction fragment. The hCTMO1 and hP67.6/CD8/ Zeta, hP67.6 / G1 / Zeta, hP67.6 / G1 / Zeta-CD28 chimeric receptor constructs were cloned into ee6HCMVNe and the hCTMO1 / CD8 /CD28, hCTMO1 Zeta-CD28 fusion and hP67.6 /h/ CD28 chimeric receptor constructs were cloned into ee6HCMVGpt.

Plasmids were linearised and transfected into Jurkat E6.1 cells (ECACC) by electroporation using a Bio-Rad Gene Pulser using the method of Rigley *et al* (J. Immunol. (1995) 154, 1136-1145). Chimeric receptor expressing colonies were selected in media either containg the drug G418 for Neo vectors or Mycophenolic acid for Gpt vectors. After approximately four weeks colonies were visible. Colonies were screened by analysis of surface expression of single chain Fv.

ANTIBODIES

Anti-idiotype antibodies are purified antisera from rabbits immunised with hCTMO1 or hP67.6. Anti-Id antibodies were purified initially on Protein A-Sepharose, absorbed out against human IgG-Sepharose and finally affinity purified on hCTMO1 or hP67.6-Sepharose. OKT3 recognises an extracellular compononent of human CD3 ε (ATCC). Anti-CD28 used in these experiments was a rat IgG2b monoclonal antibody (clone YTH 913.12) directed against the extracellular component of human CD28 (Cymbus Bioscience). FITC labelled donkey anti-rabbit Ig recognises rabbit heavy and light chains (Jackson Research Laboratories).

ANALYSIS OF SURFACE EXPRESSION OF scFv

Approximately $5X10^5$ cells were stained with saturating concentrations of anti-idiotype (10μg/ml), then incubated with fluorescein-conjugated donkey anti-rabbit antibody. Fluorescence was analysed by FACScan (Beckton Dickinson).

ACTIVATION ASSAYS

a) Anti-Id stimulation

$1 X 10^6$ Jurkat transfectants were incubated in a 96 well plate (Nunc) previously coated with / without a saturating concentration of anti-idiotype antibody at 37°C / 5% CO_2 in non-selective media. Additional stimuli of anti-CD28 and OKT3 were added in solution to a final concentration of 5μg/mL. After 18 to 20 hours cells were centrifuged and supernatant assayed for human IL-2 (Quantikine kit, R & D Systems).

b) Antigen expressing cell stimulation

1 X 10^6 Jurkat transfectants were incubated with 1 X 10^5 MCF-7 cells (P.E.M. antigen expressing) in a 96 well plate (Falcon) overnight at 37°C / 5% CO_2.

Additional stimulus of anti-CD28 was added in solution to a final concentration of 5µg/mL. After 18 to 20 hours cells were centrifuged and supernatant assayed for human IL-2 (Quantikine kit, R & D Systems).

RESULTS

Cross-linking the T-cell receptor with anti-CD3 antibodies can be used to stimulate T-cell lines such as Jurkat E6.1 to produce cytokines including IL-2. The expression of IL-2 can be further enhanced by co-stimulation by means of antibodies to the CD28 cell surface molecule in this cell line. This therefore provides a convenient model system to evaluate chimeric receptors for the ability to deliver signals which are co-stimulatory for T-cell activation.

1. Enhancement of IL2 production by a Jurkat E6.1 cell line transfected with an hCTM01 scFv-CD8- TCR ζ chimeric receptor (plasmid pTB3 in response to antigen or anti-idiotype antibody by co-stimulation with an anti-CD28 antibody.

The cell lines TB 3.2, 3.13 and 3.24 were stable cell lines derived from Jurkat E6.1 transfected with TM01hCscFv/CD8/Zeta. Figure 10 shows IL2 production by these cell lines when stimulated with an anti-CTMO1 idiotypic antibody alone or in combination with an anti-CD28 antibody. In each case the co-stimulation with anti CD-28 results in a greater than 2-fold stimulation of IL2 production compared to stimulation with anti-CTM01 idiotype antibody alone. Incubation of these cell lines with anti-CD28 alone did not result in stimulation of IL2.

Figure 11 shows the production of IL2 by one of the above cell lines (TB 3.13) when stimulated with antigen expressing tumour cells. As in figure 10 this is shown with and without co-stimulation using anti-CD28 antibody and indicates that co-stimulation can enhance IL-2 production when stimulation of the chimeric receptor is mediated by antigen.

2. Construction and testing of a chimeric receptor designed to generate a response analogous to CD28 stimulation on interaction with the extracellular scfv component.

Having established that co-stimulation via the CD28 molecule could result in enhancement of the response of a T cell transfectant to a tumour associated antigen a chimeric receptor incorporating the CD28 transmembrane and cytoplasmic components was constructed. This hCTM01/CD8/CD28 chimeric receptor (pHMF332) (HGT1) was transfected into Jurkat E6.1 cells to generate stable cell lines. Two of these lines HGT 1.2 and 1.4 were incubated in the presence of various combinations of stimulating antibodies as shown in figure 12 (see materials and methods for experimental procedure), and anti-idiotypic antibody was used to stimulate the chimeric receptor.

Incubation of the cell lines shown with an anti-CD3 antibody resulted in a low level of IL2 production. This stimulation could be enhanced by co-stimulating with an anti-CD28 antibody (column 5 figs. 12a and 12b).

Incubation with the anti-CD28 alone as expected did not result in IL2 production.

Similarly incubation with the anti-idiotypic antibody alone (stimulating the chimeric CD28 receptor) resulted in no IL2 production. However, by analogy with the combined anti-CD3 and anti-CD28 stimulation, incubation with anti-CD3 and anti-idiotype resulted in IL2 production enhanced over CD3 stimulation alone. This demonstrates that a chimeric receptor could be constructed that responds via stimulation of extracellular scFv to generate an intracellular signal capable of costimulating CD3 mediated activation.

3. Provision of both primary and accessory stimulation in the same effector cell.

In order to provide both primary (for example TCR ζ mediated) and co-stimulatory (for example CD28 mediated) activation of the effector cell via interaction of a chimeric receptor with a defined ligand or antigen a fusion

receptor incorporating two different signalling components was constructed. This chimeric receptor hCTM01/CD8/TCRZeta-CD28 (pHMF334) was transfected into Jurkat E6.1 cells and stable lines selected. One of these lines (HGT 2.4) was incubated with various combinations of antibodies and IL2 production measured (see Fig. 13).

The anti-CD3 and anti-CD28 antibodies individually and in combination resulted in a similar relative stimulation of IL2 production to that seen with the other transfected cell lines. However, with the construct HGT2 the anti-idiotype antibody alone resulted in a level of IL2 production greater than achieved with the combined anti-CD3 and anti-CD28 antibodies. Furthermore, the stimulation achieved with the single anti-idiotypic interaction could not be enhanced by further co-stimulation with anti-CD3, anti-CD28 or combinations of these.

Table 1 shows a number of preferred recombinant chimeric receptors which may be made in an analogous way by following the above teaching and methods.

Table 2 gives details of the chimeric receptor constructs and cell line nomenclature used.

POSSIBLE CHIMERIC RECEPTOR COMBINATIONS TABLE 1

	LIGAND BINDING	SPACER	TRANS MEMBRANE	SPACER	CYTOSOLIC COMPONENT	SPACER	CYTOSOLIC COMPONENT	SPACER	CYTOSOL* SPACERS
A	TAA SCFV	G1	TCR ZETA	OPT**	TCR ZETA	OPT	OPT	OPT	OPT
	TAA SCFV	h	CD28	OPT	CD28	OPT	OPT	OPT	OPT
B	TAA SCFV	CD8	TCR ZETA	OPT	TCR ZETA	OPT	OPT	OPT	OPT
	TAA SCFV	h	CD28	OPT	CD28	OPT	OPT	OPT	OPT
C	TAA SCFV	G1	TCR ZETA	OPT	TCR ZETA	OPT	OPT	OPT	OPT
	TAA SCFV	G1	IL2 R β	OPT	IL2 R β	OPT	IL2 R γ	OPT	OPT
D	TAA SCFV	G1	TCR ZETA	OPT	TCR ZETA	OPT	CD28	OPT	OPT
E	TAA SCFV	h	TCR ZETA	OPT	TCR ZETA	OPT	CD28	OPT	OPT
F	TAA SCFV	G1	TCR ZETA	OPT	TCR ZETA	OPT	IL2 R β	OPT	IL2 R γ

A,B and C describe pairs of genes coding for pairs of chimeric receptors
D,E and F describe fusion chimeric receptors, as shown in C one of a pair of receptors may be a fusion receptor

TAA SCFV denotes a single chain FV to a Tumour associated antigen
For a pair of chimeric receptors the SCFVs may bind the same or different epitopes of the same antigen or different antigens on the same or different cells.

G1 is the IgG CH_3 CH_2 HINGE spacer construct described in the text
h denotes theIgG hinge plus part of the CD28 extracelluar component described in the text

* one or more further cytosolic and or spacer components

** OPT = optional

CHIMERIC RECEPTOR CONSTRUCTS AND CELL LINE NOMENCLATURE

CONSTRUCT	EXPRESSION PLASMID	CELL LINES
hCTMO1 scFv / CD8 / TCR zeta	pTB3	TB3.
hP67.6 scFv / CD8 / TCR zeta	pTB5	TB5.
hCTMO1 scFv / CD8 / CD28	pHMF332	HGT1.
hCTMO1 scFv / CD8 / TCR zeta CD28 fusion	pHMF 334	HGT 2
hP67.6 scFv / G1 / TCRzeta	pHMF 351	HGT6
hP67.6 / G1 / TCR zeta CD28	pHMF 355	HGT7
hP67.6 / h / CD28	pHMF 353	HGT 8 and HGT 14

G1 is the IgG CH_3 CH_2 hinge spacer

(h) is the IgG hinge component plus part of CD28 extracellular domain

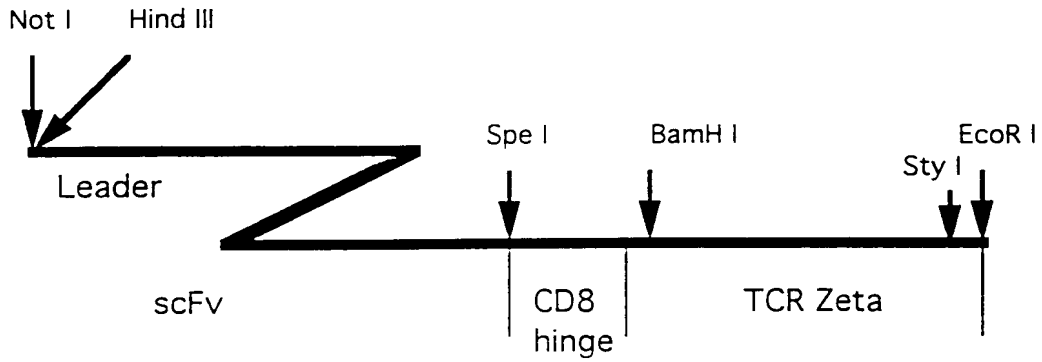
Constructs pTB 3 and 5, pHMF 334 , 351 and 355 include the TCR zeta transmembrane domain

Constructs pHMF 332 and 353 include the CD28 transmembrane domain

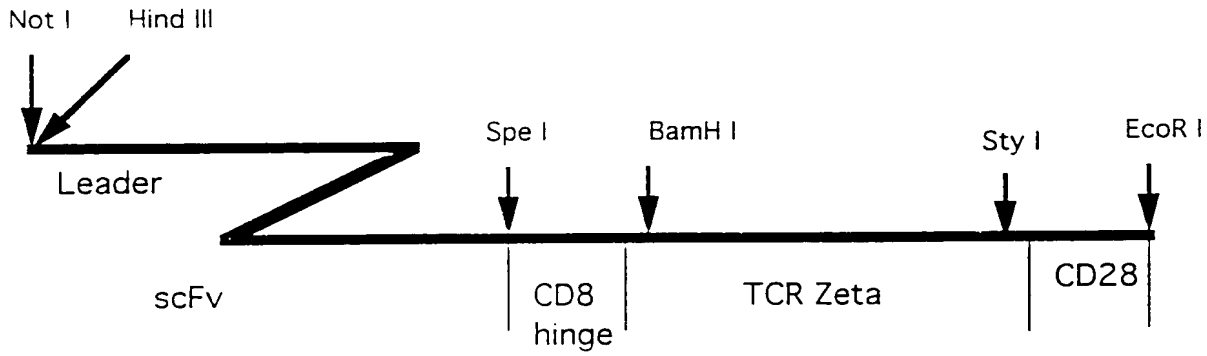
TABLE 2

Figure 1: Construct cassettes cloned into pBluescript KS +

scFv / CD8 /Zeta T-body



scFv / CD8 / Zeta-CD28 fusion T-body



scFv / CD8 / CD28 T-body

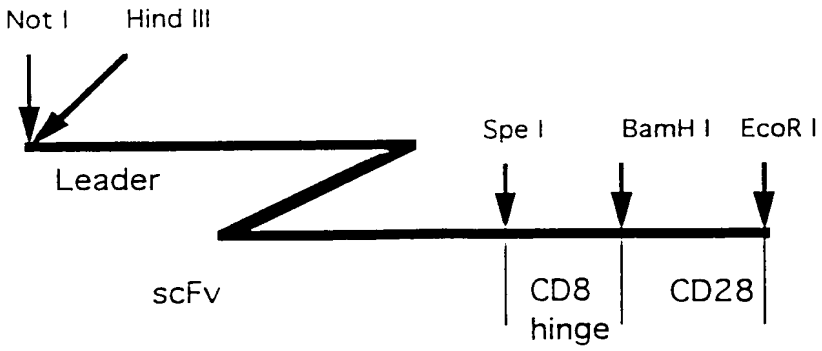
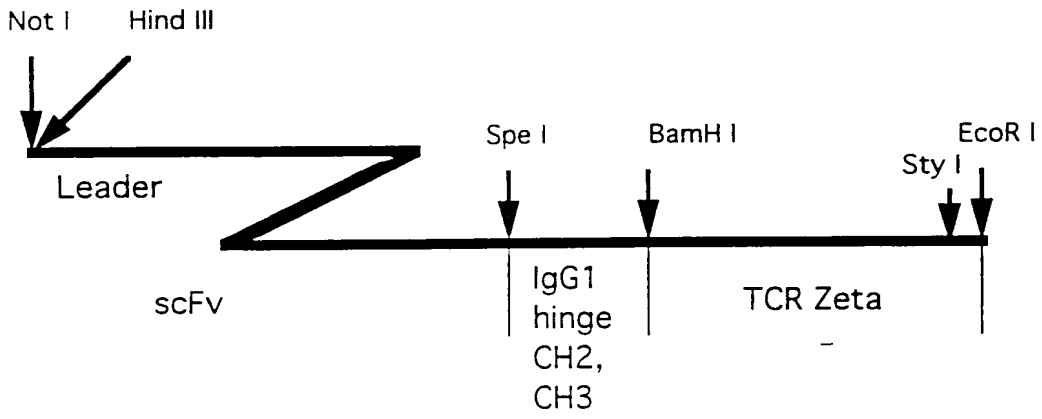
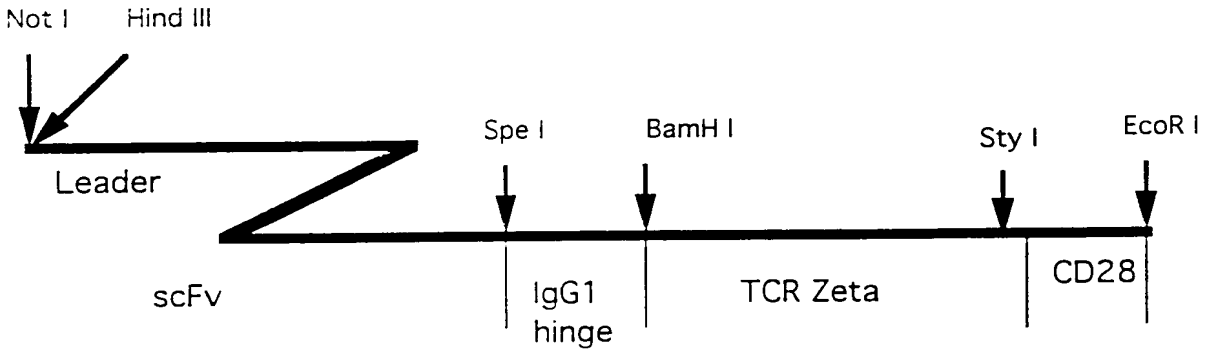


Figure 2: Construct cassettes cloned into pBluescript KS +

scFv / G1 /Zeta T-body



scFv / G1 / Zeta-CD28 fusion T-body



scFv / h / CD28 T-body

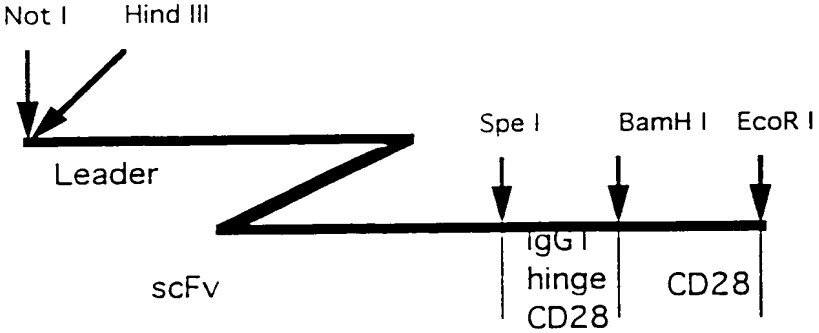


FIGURE 3 :
OLIGONUCLEOTIDE SEQUENCES FOR CHIMERIC RECEPTOR CONSTRUCTION

All oligos listed in the 5' to 3' orientation.

R6490 : ATA TAG CGG CCG CAA GCT TCC ACC ATG TCT GTC CCC ACC CAA GTC CTC

R6516 : TGA CCC TCC GCC ACC TGA CCC TCC GCC ACC TGA CCC TCC GCC ACC TGA CCC TCC GCC ACC CGT ACG TTT TAC TTC TAC TTT

R6515 : GGT GGC GGA GGG TCA GGT GGC GGA GGG TCA GGT GGC GGA GGG TCA GGT GGC GGA GGG TCA CAG ATT CAG CTG GTG CAG TCT

R6514 : TAT ATA CTA GTC GGG CCC TTC GTT GAG GCA

R6494 : ATA TAA CTA GTA ACT CCA TCA TGT ACT TCA GCC ACT TCG TGC CGG TCT TCC TGC CAG CG

R6495 : CGG TGT TGG TGG TCG CGG CGC TGG CGT CGT GGT GGG CTT CGC TGG CAG GAA GAC CGG CAC

R6496 : GCG CCG CGA CCA CCA ACA CCG GCG CCC ACC ATC GCG TCG CAG CCC CTG TCC CTG CGC CCA

R6497 : TAT ATG GAT CCA GCA GGC CAA AGC TCT GCG CCT CTG GGC GCA GGG ACA GGG GCT G

R6506 : TAT ATG GAT CCC GCC TCT GGG CGC AGG GAC AGG GGC TG

R6488 : ATA TAG GAT CCC AAA CTC TGC TAC CTG CTG

R6489 : TAT ATG AAT TCT TAG CGA GGG GGC AGG GCC TGC AT

P3240 : TAT GGA TCC AAG CCC TTT TGG GTG CTG GTG GTG

P3241 : TAT GAA TTC TCA GGA GCG ATA GGC TGC GAA

P3301 : GCC ACC AAG GAC ACC TAC GAC GC

P3302 : CCC CCT CGC AGG AGT AAG AGG AGC AGG CTC CTG CAC AGT GAC TAC ATG AAC ATG ACT CCC C

P3303 : CAA GCA TTA CCA GCC CTA TGC CCC ACC ACG CGA CTT CGC AGC CTA TCG CTC CTG AGA ATT CAT A

P3304 : TAT GAA TTC TCA GGA GCG ATA G

P3305 : GCA TAG GGCTGG TAA TGC TTG CGG GTG GGC CCG GGG CGG CGG GGA GTC ATG TTC ATG TAG T

P3306 : CTC TTA CTC CTG CGA GGG GGC AGG GCC TGC ATG TGA AGG GCG TCG TAG GTG TCC TTG GTG GC

S0146 : CGA CTA GTG ACA AAA CTC ACA CAT GCC CAC CGT GCC CAA AAG GGA AAC ACC TTT GTC CAA GGT CCC

S0060 : CGA CTA GTG ACA AAA CTC ACA CAT GCC CAC CG

S0061 : TTG GGA TCC AGT TTA CCC GGA GAC AGG GAG AGG CT

SEQUENCE OF hCTM01 / CD8 /-ZETA RECOMBINANT CHIMERIC RECEPTOR

FIGURE 4

```
                10          20          30          40
                *           *           *           *
ATG TCT GTC CCC ACC CAA GTC CTC GGA CTC CTG CTG CTG TGG
TAC AGA CAG GGG TGG GTT CAG GAG CCT GAG GAC GAC GAC ACC
 M   S   V   P   T   Q   V   L   G   L   L   L   L  W>

        50          60          70          80
        *           *           *           *
CTT ACA GAT GCC AGA TGC GAT ATC CAG ATG ACT CAG AGT CCA
GAA TGT CTA CGG TCT ACG CTA TAG GTC TAC TGA GTC TCA GGT
 L   T   D   A   R   C   D   I   Q   M   T   Q   S  P>

     90          100         110         120
     *           *           *           *
AGT ACT CTC AGT GCC AGT GTA GGT GAT AGG GTC ACC ATC ACT
TCA TGA GAG TCA CGG TCA CAT CCA CTA TCC CAG TGG TAG TGA
 S   T   L   S   A   S   V   G   D   R   V   T   I  T>

  130         140         150         160
  *           *           *           *
TGT AGG AGT AGT AAA AGT CTC CTC CAT AGT AAC GGT GAC ACC
ACA TCC TCA TCA TTT TCA GAG GAG GTA TCA TTG CCA CTG TGG
 C   R   S   S   K   S   L   L   H   S   N   G   D  T>

170         180         190         200         210
*           *           *           *           *
TTC CTC TAT TGG TTC CAG CAG AAA CCA GGT AAA GCC CCA AAG
AAG GAG ATA ACC AAG GTC GTC TTT GGT CCA TTT CGG GGT TTC
 F   L   Y   W   F   Q   Q   K   P   G   K   A   P  K>

            220         230         240         250
            *           *           *           *
CTC CTC ATG TAT AGG ATG AGT AAC CTC GCC AGT GGT GTA CCA
GAG GAG TAC ATA TCC TAC TCA TTG GAG CGG TCA CCA CAT GGT
 L   L   M   Y   R   M   S   N   L   A   S   G   V  P>

        260         270         280         290
        *           *           *           *
TCT AGA TTC AGT GGT AGT GGT AGT GGT ACT GAG TTC ACT CTC
AGA TCT AAG TCA CCA TCA CCA TCA CCA TGA CTC AAG TGA GAG
 S   R   F   S   G   S   G   S   G   T   E   F   T  L>

    300         310         320         330
    *           *           *           *
ACT ATC AGT AGT CTC CAG CCA GAT GAT TTC GCC ACT TAT TAT
TGA TAG TCA TCA GAG GTC GGT CTA CTA AAG CGG TGA ATA ATA
 T   I   S   S   L   Q   P   D   D   F   A   T   Y  Y>

  340         350         360         370
  *           *           *           *
TGT ATG CAG CAT CTC GAA TAT CCA TTC ACT TTC GGT CAG GGT
ACA TAC GTC GTA GAG CTT ATA GGT AAG TGA AAG CCA GTC CCA
 C   M   Q   H   L   E   Y   P   F   T   F   G   Q  G>

380         390         400         410         420
*           *           *           *           *
ACT AAA GTA GAA GTA AAA CGT ACG GGT GGC GGA GGG TCA GGT
TGA TTT CAT CTT CAT TTT GCA TGC CCA CCG CCT CCC AGT CCA
 T   K   V   E   V   K   R   T   G   G   G   G   S  G>

            430         440         450         460
            *           *           *           *
GGC GGA GGG TCA GGT GGC GGA GGG TCA GGT GGC GGA GGG TCA
CCG CCT CCC AGT CCA CCG CCT CCC AGT CCA CCG CCT CCC AGT
 G   G   G   S   G   G   G   G   S   G   G   G   G  S>
```

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       470          480          490          500
        *            *            *            *
GGT GGC GGA GGG TCA CAG ATT CAG CTG GTG CAG TCT GGA GCA
CCA CCG CCT CCC AGT GTC TAA GTC GAC CAC GTC AGA CCT CGT
 G   G   G   G   S   Q   I   Q   L   V   Q   S   G   A>

    510          520          530          540
     *            *            *            *
GAG GTG AAG AAG CCT GGA TCT TCT GTG AAG GTG TCT TGT AAG
CTC CAC TTC TTC GGA CCT AGA AGA CAC TTC CAC AGA ACA TTC
 E   V   K   K   P   G   S   S   V   K   V   S   C   K>

  550          560          570          580
   *            *            *            *
GCA TCT GGA TAC ACC TTC ACC GAC TAC TAC ATT AAT TGG ATG
CGT AGA CCT ATG TGG AAG TGG CTG ATG ATG TAA TTA ACC TAC
 A   S   G   Y   T   F   T   D   Y   Y   I   N   W   M>

590          600          610          620          630
 *            *            *            *            *
AGA CAG GCA CCT GGA CAG GGA CTC GAG TGG ATT GGA TGG ATT
TCT GTC CGT GGA CCT GTC CCT GAG CTC ACC TAA CCT ACC TAA
 R   Q   A   P   G   Q   G   L   E   W   I   G   W   I>

          640          650          660          670
           *            *            *            *
GAC CCT GGA TCT GGA AAT ACA AAG TAC AAT GAG AAG TTC AAG
CTG GGA CCT AGA CCT TTA TGT TTC ATG TTA CTC TTC AAG TTC
 D   P   G   S   G   N   T   K   Y   N   E   K   F   K>

       680          690          700          710
        *            *            *            *
GGA AGA GCA ACA CTG ACA GTG GAC ACA TCC ACG AAT ACC GCC
CCT TCT CGT TGT GAC TGT CAC CTG TGT AGG TGC TTA TGG CGG
 G   R   A   T   L   T   V   D   T   S   T   N   T   A>

    720          730          740          750
     *            *            *            *
TAC ATG GAG CTG TCT TCT CTG AGA TCT GAG GAC ACA GCA TTC
ATG TAC CTC GAC AGA AGA GAC TCT AGA CTC CTG TGT CGT AAG
 Y   M   E   L   S   S   L   R   S   E   D   T   A   F>

  760          770          780          790
   *            *            *            *
TAC TTC TGT GCA AGA GAG AAG ACC ACC TAC TAC TAC GCA ATG
ATG AAG ACA CGT TCT CTC TTC TGG TGG ATG ATG ATG CGT TAC
 Y   F   C   A   R   E   K   T   T   Y   Y   Y   A   M>

800          810          820          830          840
 *            *            *            *            *
GAC TAC TGG GGA CAG GGA ACA CTG GTG ACA GTG TCT TCT GCC
CTG ATG ACC CCT GTC CCT TGT GAC CAC TGT CAC AGA AGA CGG
 D   Y   W   G   Q   G   T   L   V   T   V   S   S   A>

          850          860          870          880
           *            *            *            *
TCA ACG AAG GGC CCG ACT AGT AAC TCC ATC ATG TAC TTC AGC
AGT TGC TTC CCG GGC TGA TCA TTG AGG TAG TAC ATG AAG TCG
 S   T   K   G   P   T   S   N   S   I   M   Y   F   S>

       890          900          910          920
        *            *            *            *
CAC TTC GTG CCG GTC TTC CTG CCA GCG AAG CCC ACC ACG ACG
GTG AAG CAC GGC CAG AAG GAC GGT CGC TTC GGG TGG TGC TGC
 H   F   V   P   V   F   L   P   A   K   P   T   T   T>
```

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    930           940           950           960
     *             *             *             *
CCA GCG CCG CGA CCA CCA ACA CCG GCG CCC ACC ATC GCG TCG
GGT CGC GGC GCT GGT GGT TGT GGC CGC GGG TGG TAG CGC AGC
 P   A   P   R   P   P   T   P   A   P   T   I   A   S>

  970           980           990          1000
    *             *             *             *
CAG CCC CTG TCC CTG CGC CCA GAG GCG CAG AGC TTT GGC CTG
GTC GGG GAC AGG GAC GCG GGT CTC CGC GTC TCG AAA CCG GAC
 Q   P   L   S   L   R   P   E   A   Q   S   F   G   L>

1010          1020          1030          1040          1050
  *             *             *             *             *
CTG GAT CCC AAA CTC TGC TAC CTG CTG GAT GGA ATC CTC TTC
GAC CTA GGG TTT GAG ACG ATG GAC GAC CTA CCT TAG GAG AAG
 L   D   P   K   L   C   Y   L   L   D   G   I   L   F>

          1060          1070          1080          1090
            *             *             *             *
ATC TAT GGT GTC ATT CTC ACT GCC TTG TTC CTG AGA GTG AAG
TAG ATA CCA CAG TAA GAG TGA CGG AAC AAG GAC TCT CAC TTC
 I   Y   G   V   I   L   T   A   L   F   L   R   V   K>

        1100          1110          1120          1130
          *             *             *             *
TTC AGC AGG AGC GCA GAG CCC CCC GCG TAC CAG CAG GGC CAG
AAG TCG TCC TCG CGT CTC GGG GGG CGC ATG GTC GTC CCG GTC
 F   S   R   S   A   E   P   P   A   Y   Q   Q   G   Q>

    1140          1150          1160          1170
      *             *             *             *
AAC CAG CTC TAT AAC GAG CTC AAT CTA GGA CGA AGA GAG GAG
TTG GTC GAG ATA TTG CTC GAG TTA GAT CCT GCT TCT CTC CTC
 N   Q   L   Y   N   E   L   N   L   G   R   R   E   E>

  1180          1190          1200          1210
    *             *             *             *
TAC GAT GTT TTG GAC AAG AGA CGT GGC CGG GAC CCT GAG ATG
ATG CTA CAA AAC CTG TTC TCT GCA CCG GCC CTG GGA CTC TAC
 Y   D   V   L   D   K   R   R   G   R   D   P   E   M>

1220          1230          1240          1250          1260
  *             *             *             *             *
GGG GGA AAG CCG AGA AGG AAG AAC CCT CAG GAA GGC CTG TAC
CCC CCT TTC GGC TCT TCC TTC TTG GGA GTC CTT CCG GAC ATG
 G   G   K   P   R   R   K   N   P   Q   E   G   L   Y>

          1270          1280          1290          1300
            *             *             *             *
AAT GAA CTG CAG AAA GAT AAG ATG GCG GAG GCC TAC AGT GAG
TTA CTT GAC GTC TTT CTA TTC TAC CGC CTC CGG ATG TCA CTC
 N   E   L   Q   K   D   K   M   A   E   A   Y   S   E>

        1310          1320          1330          1340
          *             *             *             *
ATT GGG ATG AAA GGC GAG CGC CGG AGG GGC AAG GGG CAC GAT
TAA CCC TAC TTT CCG CTC GCG GCC TCC CCG TTC CCC GTG CTA
 I   G   M   K   G   E   R   R   R   G   K   G   H   D>

    1350          1360          1370          1380
      *             *             *             *
GGC CTT TAC CAG GGT CTC AGT ACA GCC ACC AAG GAC ACC TAC
CCG GAA ATG GTC CCA GAG TCA TGT CGG TGG TTC CTG TGG ATG
 G   L   Y   Q   G   L   S   T   A   T   K   D   T   Y>
```

```
1390          1400          1410          1420
   *             *             *             *
GAC GCC CTT CAC ATG CAG GCC CTG CCC CCT CGC TAA
CTG CGG GAA GTG TAC GTC CGG GAC GGG GGA GCG ATT
 D   A   L   H   M   Q   A   L   P   P   R   *
```

FIGURE 5

SEQUENCE OF hCTM01 / CD8 / Zeta-CD28 "FUSION RECOMBINANT CHIMERIC RECEPTOR

```
          10          20          30          40
           *           *           *           *
ATG TCT GTC CCC ACC CAA GTC CTC GGA CTC CTG CTG CTG TGG CTT ACA
TAC AGA CAG GGG TGG GTT CAG GAG CCT GAG GAC GAC GAC ACC GAA TGT
 m   s   v   p   t   q   v   l   g   l   l   l   l   w   l   t>

50          60          70          80          90
 *           *           *           *           *
GAT GCC AGA TGC GAT ATC CAG ATG ACT CAG AGT CCA AGT ACT CTC AGT
CTA CGG TCT ACG CTA TAG GTC TAC TGA GTC TCA GGT TCA TGA GAG TCA
 d   a   r   c   D   I   Q   M   T   Q   S   P   S   T   L   S>

  100         110         120         130         140
   *           *           *           *           *
GCC AGT GTA GGT GAT AGG GTC ACC ATC ACT TGT AGG AGT AGT AAA AGT
CGG TCA CAT CCA CTA TCC CAG TGG TAG TGA ACA TCC TCA TCA TTT TCA
 A   S   V   G   D   R   V   T   I   T   C   R   S   S   K   S>

    150         160         170         180         190
     *           *           *           *           *
CTC CTC CAT AGT AAC GGT GAC ACC TTC CTC TAT TGG TTC CAG CAG AAA
GAG GAG GTA TCA TTG CCA CTG TGG AAG GAG ATA ACC AAG GTC GTC TTT
 L   L   H   S   N   G   D   T   F   L   Y   W   F   Q   Q   K>

        200         210         220         230         240
         *           *           *           *           *
CCA GGT AAA GCC CCA AAG CTC CTC ATG TAT AGG ATG AGT AAC CTC GCC
GGT CCA TTT CGG GGT TTC GAG GAG TAC ATA TCC TAC TCA TTG GAG CGG
 P   G   K   A   P   K   L   L   M   Y   R   M   S   N   L   A>

          260         260         270         280
           *           *           *           *
AGT GGT GTA CCA TCT AGA TTC AGT GGT AGT GGT AGT GGT ACT GAG TTC
TCA CCA CAT GGT AGA TCT AAG TCA CCA TCA CCA TCA CCA TGA CTC AAG
 S   G   V   P   S   R   F   S   G   S   G   S   G   T   E   F>

290         300         310         320         330
 *           *           *           *           *
ACT CTC ACT ATC AGT AGT CTC CAG CCA GAT GAT TTC GCC ACT TAT TAT
TGA GAG TGA TAG TCA TCA GAG GTC GGT CTA CTA AAG CGG TGA ATA ATA
 T   L   T   I   S   S   L   Q   P   D   D   F   A   T   Y   Y>

  340         350         360         370         380
   *           *           *           *           *
TGT ATG CAG CAT CTC GAA TAT CCA TTC ACT TTC GGT CAG GGT ACT AAA
ACA TAC GTC GTA GAG CTT ATA GGT AAG TGA AAG CCA GTC CCA TGA TTT
 C   M   Q   H   L   E   Y   P   F   T   F   G   Q   G   T   K>

    390         400         410         420         430
     *           *           *           *           *
GTA GAA GTA AAA CGT ACG GGT GGC GGA GGG TCA GGT GGC GGA GGG TCA
CAT CTT CAT TTT GCA TGC CCA CCG CCT CCC AGT CCA CCG CCT CCC AGT
 V   E   V   K   R   T   G   G   G   G   S   G   G   G   G   S>

        440         450         460         470         480
         *           *           *           *           *
GGT GGC GGA GGG TCA GGT GGC GGA GGG TCA GGT GGC GGA GGG TCA CAG
CCA CCG CCT CCC AGT CCA CCG CCT CCC AGT CCA CCG CCT CCC AGT GTC
 G   G   G   G   S   G   G   G   G   S   G   G   G   G   S   Q>

          490         500         510         520
           *           *           *           *
ATT CAG CTG GTG CAG TCT GGA GCA GAG GTG AAG AAG CCT GGA TCT TCT
TAA GTC GAC CAC GTC AGA CCT CGT CTC CAC TTC TTC GGA CCT AGA AGA
 I   Q   L   V   Q   S   G   A   E   V   K   K   P   G   S   S>
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```
530          540          550          560          570
 *            *            *            *            *
GTG AAG GTG TCT TGT AAG GCA TCT GGA TAC ACC TTC ACC GAC TAC TAC
CAC TTC CAC AGA ACA TTC CGT AGA CCT ATG TGG AAG TGG CTG ATG ATG
 V   K   V   S   C   K   A   S   G   Y   T   F   T   D   Y   Y>

  580          590          600          610          620
   *            *            *            *            *
ATT AAT TGG ATG AGA CAG GCA CCT GGA CAG GGA CTC GAG TGG ATT GGA
TAA TTA ACC TAC TCT GTC CGT GGA CCT GTC CCT GAG CTC ACC TAA CCT
 I   N   W   M   R   Q   A   P   G   Q   G   L   E   W   I   G>

    630          640          650          660          670
     *            *            *            *            *
TGG ATT GAC CCT GGA TCT GGA AAT ACA AAG TAC AAT GAG AAG TTC AAG
ACC TAA CTG GGA CCT AGA CCT TTA TGT TTC ATG TTA CTC TTC AAG TTC
 W   I   D   P   G   S   G   N   T   K   Y   N   E   K   F   K>

        680          690          700          710          720
         *            *            *            *            *
GGA AGA GCA ACA CTG ACA GTG GAC ACA TCC ACG AAT ACC GCC TAC ATG
CCT TCT CGT TGT GAC TGT CAC CTG TGT AGG TGC TTA TGG CGG ATG TAC
 G   R   A   T   L   T   V   D   T   S   T   N   T   A   Y   M>

          730          740          750          760
           *            *            *            *
GAG CTG TCT TCT CTG AGA TCT GAG GAC ACA GCA TTC TAC TTC TGT GCA
CTC GAC AGA AGA GAC TCT AGA CTC CTG TGT CGT AAG ATG AAG ACA CGT
 E   L   S   S   L   R   S   E   D   T   A   F   Y   F   C   A>

770          780          790          800          810
 *            *            *            *            *
AGA GAG AAG ACC ACC TAC TAC TAC GCA ATG GAC TAC TGG GGA CAG GGA
TCT CTC TTC TGG TGG ATG ATG ATG CGT TAC CTG ATG ACC CCT GTC CCT
 R   E   K   T   T   Y   Y   Y   A   M   D   Y   W   G   Q   G>

  820          830          840          850          860
   *            *            *            *            *
ACA CTG GTG ACA GTG TCT TCT GCC TCA ACG AAG GGC CCG ACT AGT AAC
TGT GAC CAC TGT CAC AGA AGA CGG AGT TGC TTC CCG GGC TGA TCA TTG
 T   L   V   T   V   S   S   A   S   T   K   G   P   T   S   N>

    870          880          890          900          910
     *            *            *            *            *
TCC ATC ATG TAC TTC AGC CAC TTC GTG CCG GTC TTC CTG CCA GCG AAG
AGG TAG TAC ATG AAG TCG GTG AAG CAC GGC CAG AAG GAC GGT CGC TTC
 S   I   M   Y   F   S   H   F   V   P   V   F   L   P   A   K>

        920          930          940          950          960
         *            *            *            *            *
CCC ACC ACG ACG CCA GCG CCG CGA CCA CCA ACA CCG GCG CCC ACC ATC
GGG TGG TGC TGC GGT CGC GGC GCT GGT GGT TGT GGC CGC GGG TGG TAG
 P   T   T   T   P   A   P   R   P   P   T   P   A   P   T   I>

          970          980          990          1000
           *            *            *            *
GCG TCG CAG CCC CTG TCC CTG CGC CCA GAG GCG CAG AGC TTT GGC CTG
CGC AGC GTC GGG GAC AGG GAC GCG GGT CTC CGC GTC TCG AAA CCG GAC
 A   S   Q   P   L   S   L   R   P   E   A   Q   S   F   G   L>

1010         1020         1030         1040         1050
 *            *            *            *            *
CTG GAT CCC AAA CTC TGC TAC CTG CTG GAT GGA ATC CTC TTC ATC TAT
GAC CTA GGG TTT GAG ACG ATG GAC GAC CTA CCT TAG GAG AAG TAG ATA
 L   D   P   K   L   C   Y   L   L   D   G   I   L   F   I   Y>
```

```
  1060        1070        1080        1090        1100
    *           *           *           *           *
GGT GTC ATT CTC ACT GCC TTG TTC CTG AGA GTG AAG TTC AGC AGG AGC
CCA CAG TAA GAG TGA CGG AAC AAG GAC TCT CAC TTC AAG TCG TCC TCG
 G   V   I   L   T   A   L   F   L   R   V   K   F   S   R   S>

  1110        1120        1130        1140        1150
    *           *           *           *           *
GCA GAG CCC CCC GCG TAC CAG CAG GGC CAG AAC CAG CTC TAT AAC GAG
CGT CTC GGG GGG CGC ATG GTC GTC CCG GTC TTG GTC GAG ATA TTG CTC
 A   E   P   P   A   Y   Q   Q   G   Q   N   Q   L   Y   N   E>

      1160        1170        1180        1190        1200
        *           *           *           *           *
CTC AAT CTA GGA CGA AGA GAG GAG TAC GAT GTT TTG GAC AAG AGA CGT
GAG TTA GAT CCT GCT TCT CTC CTC ATG CTA CAA AAC CTG TTC TCT GCA
 L   N   L   G   R   R   E   E   Y   D   V   L   D   K   R   R>

          1210        1220        1230        1240
            *           *           *           *
GGC CGG GAC CCT GAG ATG GGG GGA AAG CCG AGA AGG AAG AAC CCT CAG
CCG GCC CTG GGA CTC TAC CCC CCT TTC GGC TCT TCC TTC TTG GGA GTC
 G   R   D   P   E   M   G   G   K   P   R   R   K   N   P   Q>

1250        1260        1270        1280        1290
  *           *           *           *           *
GAA GGC CTG TAC AAT GAA CTG CAG AAA GAT AAG ATG GCG GAG GCC TAC
CTT CCG GAC ATG TTA CTT GAC GTC TTT CTA TTC TAC CGC CTC CGG ATG
 E   G   L   Y   N   E   L   Q   K   D   K   M   A   E   A   Y>

  1300        1310        1320        1330        1340
    *           *           *           *           *
AGT GAG ATT GGG ATG AAA GGC GAG CGC CGG AGG GGC AAG GGG CAC GAT
TCA CTC TAA CCC TAC TTT CCG CTC GCG GCC TCC CCG TTC CCC GTG CTA
 S   E   I   G   M   K   G   E   R   R   R   G   K   G   H   D>

    1350        1360        1370        1380        1390
      *           *           *           *           *
GGC CTT TAC CAG GGT CTC AGT ACA GCC ACC AAG GAC ACC TAC GAC GCC
CCG GAA ATG GTC CCA GAG TCA TGT CGG TGG TTC CTG TGG ATG CTG CGG
 G   L   Y   Q   G   L   S   T   A   T   K   D   T   Y   D   A>

      1400        1410        1420        1430        1440
        *           *           *           *           *
CTT CAC ATG CAG GCC CTG CCC CCT CGC AGG AGT AAG AGG AGC AGG CTC
GAA GTG TAC GTC CGG GAC GGG GGA GCG TCC TCA TTC TCC TCG TCC GAG
 L   H   M   Q   A   L   P   P   R   R   S   K   R   S   R   L>

          1450        1460        1470        1480
            *           *           *           *
CTG CAC AGT GAC TAC ATG AAC ATG ACT CCC CGC CGC CCC GGG CCC ACC
GAC GTG TCA CTG ATG TAC TTG TAC TGA GGG GCG GCG GGG CCC GGG TGG
 L   H   S   D   Y   M   N   M   T   P   R   R   P   G   P   T>

1490        1500        1510        1520        1530
  *           *           *           *           *
CGC AAG CAT TAC CAG CCC TAT GCC CCA CCA CGC GAC TTC GCA GCC TAT
GCG TTC GTA ATG GTC GGG ATA CGG GGT GGT GCG CTG AAG CGT CGG ATA
 R   K   H   Y   Q   P   Y   A   P   P   R   D   F   A   A   Y>

  1540
    *
CGC TCC TGA
GCG AGG ACT
 R   S   *
```

SEQUENCE OF hCTMO1 / CD8 / CD28 RECOMBINANT CHIMERIC RECEPTOR FIGURE 6

```
          10          20          30          40
           *           *           *           *
ATG TCT GTC CCC ACC CAA GTC CTC GGA CTC CTG CTG CTG TGG
TAC AGA CAG GGG TGG GTT CAG GAG CCT GAG GAC GAC GAC ACC
 M   S   V   P   T   Q   V   L   G   L   L   L   L   W>

        50          60          70          80
         *           *           *           *
CTT ACA GAT GCC AGA TGC GAT ATC CAG ATG ACT CAG AGT CCA
GAA TGT CTA CGG TCT ACG CTA TAG GTC TAC TGA GTC TCA GGT
 L   T   D   A   R   C   D   I   Q   M   T   Q   S   P>

      90          100         110         120
       *           *           *           *
AGT ACT CTC AGT GCC AGT GTA GGT GAT AGG GTC ACC ATC ACT
TCA TGA GAG TCA CGG TCA CAT CCA CTA TCC CAG TGG TAG TGA
 S   T   L   S   A   S   V   G   D   R   V   T   I   T>

    130         140         150         160
     *           *           *           *
TGT AGG AGT AGT AAA AGT CTC CTC CAT AGT AAC GGT GAC ACC
ACA TCC TCA TCA TTT TCA GAG GAG GTA TCA TTG CCA CTG TGG
 C   R   S   S   K   S   L   L   H   S   N   G   D   T>

170         180         190         200         210
 *           *           *           *           *
TTC CTC TAT TGG TTC CAG CAG AAA CCA GGT AAA GCC CCA AAG
AAG GAG ATA ACC AAG GTC GTC TTT GGT CCA TTT CGG GGT TTC
 F   L   Y   W   F   Q   Q   K   P   G   K   A   P   K>

          220         230         240         250
           *           *           *           *
CTC CTC ATG TAT AGG ATG AGT AAC CTC GCC AGT GGT GTA CCA
GAG GAG TAC ATA TCC TAC TCA TTG GAG CGG TCA CCA CAT GGT
 L   L   M   Y   R   M   S   N   L   A   S   G   V   P>

        260         270         280         290
         *           *           *           *
TCT AGA TTC AGT GGT AGT GGT AGT GGT ACT GAG TTC ACT CTC
AGA TCT AAG TCA CCA TCA CCA TCA CCA TGA CTC AAG TGA GAG
 S   R   F   S   G   S   G   S   G   T   E   F   T   L>

    300         310         320         330
     *           *           *           *
ACT ATC AGT AGT CTC CAG CCA GAT GAT TTC GCC ACT TAT TAT
TGA TAG TCA TCA GAG GTC GGT CTA CTA AAG CGG TGA ATA ATA
 T   I   S   S   L   Q   P   D   D   F   A   T   Y   Y>

  340         350         360         370
   *           *           *           *
TGT ATG CAG CAT CTC GAA TAT CCA TTC ACT TTC GGT CAG GGT
ACA TAC GTC GTA GAG CTT ATA GGT AAG TGA AAG CCA GTC CCA
 C   M   Q   H   L   E   Y   P   F   T   F   G   Q   G>

380         390         400         410         420
 *           *           *           *           *
ACT AAA GTA GAA GTA AAA CGT ACG GGT GGC GGA GGG TCA GGT
TGA TTT CAT CTT CAT TTT GCA TGC CCA CCG CCT CCC AGT CCA
 T   K   V   E   V   K   R   T   G   G   G   G   S   G>

          430         440         450         460
           *           *           *           *
GGC GGA GGG TCA GGT GGC GGA GGG TCA GGT GGC GGA GGG TCA
CCG CCT CCC AGT CCA CCG CCT CCC AGT CCA CCG CCT CCC AGT
 G   G   G   S   G   G   G   G   S   G   G   G   G   S>
```

```
       470          480          490          500
        *            *            *            *
GGT GGC GGA GGG TCA CAG ATT CAG CTG GTG CAG TCT GGA GCA
CCA CCG CCT CCC AGT GTC TAA GTC GAC CAC GTC AGA CCT CGT
 G   G   G   G   S   Q   I   Q   L   V   Q   S   G   A>

    510          520          530          540
     *            *            *            *
GAG GTG AAG AAG CCT GGA TCT TCT GTG AAG GTG TCT TGT AAG
CTC CAC TTC TTC GGA CCT AGA AGA CAC TTC CAC AGA ACA TTC
 E   V   K   K   P   G   S   S   V   K   V   S   C   K>

  550          560          570          580
   *            *            *            *
GCA TCT GGA TAC ACC TTC ACC GAC TAC TAC ATT AAT TGG ATG
CGT AGA CCT ATG TGG AAG TGG CTG ATG ATG TAA TTA ACC TAC
 A   S   G   Y   T   F   T   D   Y   Y   I   N   W   M>

590          600          610          620          630
 *            *            *            *            *
AGA CAG GCA CCT GGA CAG GGA CTC GAG TGG ATT GGA TGG ATT
TCT GTC CGT GGA CCT GTC CCT GAG CTC ACC TAA CCT ACC TAA
 R   Q   A   P   G   Q   G   L   E   W   I   G   W   I>

          640          650          660          670
           *            *            *            *
GAC CCT GGA TCT GGA AAT ACA AAG TAC AAT GAG AAG TTC AAG
CTG GGA CCT AGA CCT TTA TGT TTC ATG TTA CTC TTC AAG TTC
 D   P   G   S   G   N   T   K   Y   N   E   K   F   K>

       680          690          700          710
        *            *            *            *
GGA AGA GCA ACA CTG ACA GTG GAC ACA TCC ACG AAT ACC GCC
CCT TCT CGT TGT GAC TGT CAC CTG TGT AGG TGC TTA TGG CGG
 G   R   A   T   L   T   V   D   T   S   T   N   T   A>

    720          730          740          750
     *            *            *            *
TAC ATG GAG CTG TCT TCT CTG AGA TCT GAG GAC ACA GCA TTC
ATG TAC CTC GAC AGA AGA GAC TCT AGA CTC CTG TGT CGT AAG
 Y   M   E   L   S   S   L   R   S   E   D   T   A   F>

  760          770          780          790
   *            *            *            *
TAC TTC TGT GCA AGA GAG AAG ACC ACC TAC TAC TAC GCA ATG
ATG AAG ACA CGT TCT CTC TTC TGG TGG ATG ATG ATG CGT TAC
 Y   F   C   A   R   E   K   T   T   Y   Y   Y   A   M>

800          810          820          830          840
 *            *            *            *            *
GAC TAC TGG GGA CAG GGA ACA CTG GTG ACA GTG TCT TCT GCC
CTG ATG ACC CCT GTC CCT TGT GAC CAC TGT CAC AGA AGA CGG
 D   Y   W   G   Q   G   T   L   V   T   V   S   S   A>

          850          860          870          880
           *            *            *            *
TCA ACG AAG GGC CCG ACT AGT AAC TCC ATC ATG TAC TTC AGC
AGT TGC TTC CCG GGC TGA TCA TTG AGG TAG TAC ATG AAG TCG
 S   T   K   G   P   T   S   N   S   I   M   Y   F   S>

       890          900          910          920
        *            *            *            *
CAC TTC GTG CCG GTC TTC CTG CCA GCG AAG CCC ACC ACG ACG
GTG AAG CAC GGC CAG AAG GAC GGT CGC TTC GGG TGG TGC TGC
 H   F   V   P   V   F   L   P   A   K   P   T   T   T>
```

```
     930           940           950           960
      *             *             *             *
CCA GCG CCG CGA CCA CCA ACA CCG GCG CCC ACC ATC GCG TCG
GGT CGC GGC GCT GGT GGT TGT GGC CGC GGG TGG TAG CGC AGC
 P   A   P   R   P   P   T   P   A   P   T   I   A   S>

  970           980           990          1000
    *             *             *             *
CAG CCC CTG TCC CTG CGC CCA GAG GCG GGA TCC AAG CCC TTT
GTC GGG GAC AGG GAC GCG GGT CTC CGC CCT AGG TTC GGG AAA
 Q   P   L   S   L   R   P   E   A   G   S   K   P   F>

1010          1020          1030          1040          1050
  *             *             *             *             *
TGG GTG CTG GTG GTG GTT GGT GGA GTC CTG GCT TGC TAT AGC
ACC CAC GAC CAC CAC CAA CCA CCT CAG GAC CGA ACG ATA TCG
 W   V   L   V   V   V   G   G   V   L   A   C   Y   S>

          1060          1070          1080          1090
            *             *             *             *
TTG CTA GTA ACA GTG GCC TTT ATT ATT TTC TGG GTG AGG AGT
AAC GAT CAT TGT CAC CGG AAA TAA TAA AAG ACC CAC TCC TCA
 L   L   V   T   V   A   F   I   I   F   W   V   R   S>

      1100          1110          1120          1130
        *             *             *             *
AAG AGG AGC AGG CTC CTG CAC AGT GAC TAC ATG AAC ATG ACT
TTC TCC TCG TCC GAG GAC GTG TCA CTG ATG TAC TTG TAC TGA
 K   R   S   R   L   L   H   S   D   Y   M   N   M   T>

   1140          1150          1160          1170
     *             *             *             *
CCC CGC CGC CCC GGG CCC ACC CGC AAG CAT TAC CAG CCC TAT
GGG GCG GCG GGG CCC GGG TGG GCG TTC GTA ATG GTC GGG ATA
 P   R   R   P   G   P   T   R   K   H   Y   Q   P   Y>

 1180          1190          1200          1210
   *             *             *             *
GCC CCA CCA CGC GAC TTC GCA GCC TAT CGC TCC TGA
CGG GGT GGT GCG CTG AAG CGT CGG ATA GCG AGG ACT
 A   P   P   R   D   F   A   A   Y   R   S   *
```

FIGURE 7

SEQUENCE OF hCTMO1 / G1 / ZETA RECOMBINANT CHIMERIC RECEPTOR

```
          10          20          30          40
           *           *           *           *
ATG TCT GTC CCC ACC CAA GTC CTC GGA CTC CTG CTG CTG TGG CTT ACA
TAC AGA CAG GGG TGG GTT CAG GAG CCT GAG GAC GAC GAC ACC GAA TGT
 M   S   V   P   T   Q   V   L   G   L   L   L   L   W   L   T>

50          60          70          80          90
 *           *           *           *           *
GAT GCC AGA TGC GAT ATC CAG ATG ACT CAG AGT CCA AGT ACT CTC AGT
CTA CGG TCT ACG CTA TAG GTC TAC TGA GTC TCA GGT TCA TGA GAG TCA
 D   A   R   C   D   I   Q   M   T   Q   S   P   S   T   L   S>

 100         110         120         130         140
  *           *           *           *           *
GCC AGT GTA GGT GAT AGG GTC ACC ATC ACT TGT AGG AGT AGT AAA AGT
CGG TCA CAT CCA CTA TCC CAG TGG TAG TGA ACA TCC TCA TCA TTT TCA
 A   S   V   G   D   R   V   T   I   T   C   R   S   S   K   S>

     150         160         170         180         190
      *           *           *           *           *
CTC CTC CAT AGT AAC GGT GAC ACC TTC CTC TAT TGG TTC CAG CAG AAA
GAG GAG GTA TCA TTG CCA CTG TGG AAG GAG ATA ACC AAG GTC GTC TTT
 L   L   H   S   N   G   D   T   F   L   Y   W   F   Q   Q   K>

       200         210         220         230         240
        *           *           *           *           *
CCA GGT AAA GCC CCA AAG CTC CTC ATG TAT AGG ATG AGT AAC CTC GCC
GGT CCA TTT CGG GGT TTC GAG GAG TAC ATA TCC TAC TCA TTG GAG CGG
 P   G   K   A   P   K   L   L   M   Y   R   M   S   N   L   A>

           250         260         270         280
            *           *           *           *
AGT GGT GTA CCA TCT AGA TTC AGT GGT AGT GGT AGT GGT ACT GAG TTC
TCA CCA CAT GGT AGA TCT AAG TCA CCA TCA CCA TCA CCA TGA CTC AAG
 S   G   V   P   S   R   F   S   G   S   G   S   G   T   E   F>

290         300         310         320         330
 *           *           *           *           *
ACT CTC ACT ATC AGT AGT CTC CAG CCA GAT GAT TTC GCC ACT TAT TAT
TGA GAG TGA TAG TCA TCA GAG GTC GGT CTA CTA AAG CGG TGA ATA ATA
 T   L   T   I   S   S   L   Q   P   D   D   F   A   T   Y   Y>

 340         350         360         370         380
  *           *           *           *           *
TGT ATG CAG CAT CTC GAA TAT CCA TTC ACT TTC GGT CAG GGT ACT AAA
ACA TAC GTC GTA GAG CTT ATA GGT AAG TGA AAG CCA GTC CCA TGA TTT
 C   M   Q   H   L   E   Y   P   F   T   F   G   Q   G   T   K>

     390         400         410         420         430
      *           *           *           *           *
GTA GAA GTA AAA CGT ACG GGT GGC GGA GGG TCA GGT GGC GGA GGG TCA
CAT CTT CAT TTT GCA TGC CCA CCG CCT CCC AGT CCA CCG CCT CCC AGT
 V   E   V   K   R   T   G   G   G   G   S   G   G   G   G   S>

       440         450         460         470         480
        *           *           *           *           *
GGT GGC GGA GGG TCA GGT GGC GGA GGG TCA GGT GGC GGA GGG TCA CAG
CCA CCG CCT CCC AGT CCA CCG CCT CCC AGT CCA CCG CCT CCC AGT GTC
 G   G   G   G   S   G   G   G   G   S   G   G   G   G   S   Q>
```

```
          490           500           510           520
           *             *             *             *
ATT CAG CTG GTG CAG TCT GGA GCA GAG GTG AAG AAG CCT GGA TCT TCT
TAA GTC GAC CAC GTC AGA CCT CGT CTC CAC TTC TTC GGA CCT AGA AGA
 I   Q   L   V   Q   S   G   A   E   V   K   K   P   G   S   S>

530           540           550           560           570
 *             *             *             *             *
GTG AAG GTG TCT TGT AAG GCA TCT GGA TAC ACC TTC ACC GAC TAC TAC
CAC TTC CAC AGA ACA TTC CGT AGA CCT ATG TGG AAG TGG CTG ATG ATG
 V   K   V   S   C   K   A   S   G   Y   T   F   T   D   Y   Y>

  580           590           600           610           620
   *             *             *             *             *
ATT AAT TGG ATG AGA CAG GCA CCT GGA CAG GGA CTC GAG TGG ATT GGA
TAA TTA ACC TAC TCT GTC CGT GGA CCT GTC CCT GAG CTC ACC TAA CCT
 I   N   W   M   R   Q   A   P   G   Q   G   L   E   W   I   G>

    630           640           650           660           670
     *             *             *             *             *
TGG ATT GAC CCT GGA TCT GGA AAT ACA AAG TAC AAT GAG AAG TTC AAG
ACC TAA CTG GGA CCT AGA CCT TTA TGT TTC ATG TTA CTC TTC AAG TTC
 W   I   D   P   G   S   G   N   T   K   Y   N   E   K   F   K>

        680           690           700           710           720
         *             *             *             *             *
GGA AGA GCA ACA CTG ACA GTG GAC ACA TCC ACG AAT ACC GCC TAC ATG
CCT TCT CGT TGT GAC TGT CAC CTG TGT AGG TGC TTA TGG CGG ATG TAC
 G   R   A   T   L   T   V   D   T   S   T   N   T   A   Y   M>

          730           740           750           760
           *             *             *             *
GAG CTG TCT TCT CTG AGA TCT GAG GAC ACA GCA TTC TAC TTC TGT GCA
CTC GAC AGA AGA GAC TCT AGA CTC CTG TGT CGT AAG ATG AAG ACA CGT
 E   L   S   S   L   R   S   E   D   T   A   F   Y   F   C   A>

770           780           790           800           810
 *             *             *             *             *
AGA GAG AAG ACC ACC TAC TAC TAC GCA ATG GAC TAC TGG GGA CAG GGA
TCT CTC TTC TGG TGG ATG ATG ATG CGT TAC CTG ATG ACC CCT GTC CCT
 R   E   K   T   T   Y   Y   Y   A   M   D   Y   W   G   Q   G>

  820           830           840           850           860
   *             *             *             *             *
ACA CTG GTG ACA GTG TCT TCT GCC TCA ACG AAG GGC CCG ACT AGT GAC
TGT GAC CAC TGT CAC AGA AGA CGG AGT TGC TTC CCG GGC TGA TCA CTG
 T   L   V   T   V   S   S   A   S   T   K   G   P   T   S   D>

    870           880           890           900           910
     *             *             *             *             *
AAA ACT CAC ACA TGC CCA CCG TGC CCA GCA CCT GAA CTC CTG GGG GGA
TTT TGA GTG TGT ACG GGT GGC ACG GGT CGT GGA CTT GAG GAC CCC CCT
 K   T   H   T   C   P   P   C   P   A   P   E   L   L   G   G>

        920           930           940           950           960
         *             *             *             *             *
CCG TCA GTC TTC CTC TTC CCC CCA AAA CCC AAG GAC ACC CTC ATG ATC
GGC AGT CAG AAG GAG AAG GGG GGT TTT GGG TTC CTG TGG GAG TAC TAG
 P   S   V   F   L   F   P   P   K   P   K   D   T   L   M   I>

          970           980           990           1000
           *             *             *             *
TCC CGG ACC CCT GAG GTC ACA TGC GTG GTG GTG GAC GTG AGC CAC GAA
```

```
  AGG GCC TGG GGA CTC CAG TGT ACG CAC CAC CAC CTG CAC TCG GTG CTT
   S   R   T   P   E   V   T   C   V   V   V   D   V   S   H   E>

1010          1020          1030          1040          1050
   *             *             *             *             *
  GAC CCT GAG GTC AAG TTC AAC TGG TAC GTG GAC GGC GTG GAG GTG CAT
  CTG GGA CTC CAG TTC AAG TTG ACC ATG CAC CTG CCG CAC CTC CAC GTA
   D   P   E   V   K   F   N   W   Y   V   D   G   V   E   V   H>

   1060          1070          1080          1090          1100
      *             *             *             *             *
  AAT GCC AAG ACA AAG CCG CGG GAG GAG CAG TAC AAC AGC ACG TAC CGT
  TTA CGG TTC TGT TTC GGC GCC CTC CTC GTC ATG TTG TCG TGC ATG GCA
   N   A   K   T   K   P   R   E   E   Q   Y   N   S   T   Y   R>

     1110          1120          1130          1140          1150
        *             *             *             *             *
  GTG GTC AGC GTC CTC ACC GTC CTG CAC CAG GAC TGG CTG AAT GGC AAG
  CAC CAG TCG CAG GAG TGG CAG GAC GTG GTC CTG ACC GAC TTA CCG TTC
   V   V   S   V   L   T   V   L   H   Q   D   W   L   N   G   K>

        1160          1170          1180          1190          1200
           *             *             *             *             *
  GAG TAC AAG TGC AAG GTC TCC AAC AAA GCC CTC CCA GCC CCC ATC GAG
  CTC ATG TTC ACG TTC CAG AGG TTG TTT CGG GAG GGT CGG GGG TAG CTC
   E   Y   K   C   K   V   S   N   K   A   L   P   A   P   I   E>

           1210          1220          1230          1240
              *             *             *             *
  AAA ACC ATC TCC AAA GCC AAA GGG CAG CCC CGA GAA CCA CAG GTG TAC
  TTT TGG TAG AGG TTT CGG TTT CCC GTC GGG GCT CTT GGT GTC CAC ATG
   K   T   I   S   K   A   K   G   Q   P   R   E   P   Q   V   Y>

1250          1260          1270          1280          1290
   *             *             *             *             *
  ACC CTG CCC CCA TCC CGG GAG GAG ATG ACC AAG AAC CAG GTC AGC CTG
  TGG GAC GGG GGT AGG GCC CTC CTC TAC TGG TTC TTG GTC CAG TCG GAC
   T   L   P   P   S   R   E   E   M   T   K   N   Q   V   S   L>

   1300          1310          1320          1330          1340
      *             *             *             *             *
  ACC TGC CTG GTC AAA GGC TTC TAT CCC AGC GAC ATC GCC GTG GAG TGG
  TGG ACG GAC CAG TTT CCG AAG ATA GGG TCG CTG TAG CGG CAC CTC ACC
   T   C   L   V   K   G   F   Y   P   S   D   I   A   V   E   W>

     1350          1360          1370          1380          1390
        *             *             *             *             *
  GAG AGC AAT GGG CAG CCG GAG AAC AAC TAC AAG ACC ACG CCT CCC GTG
  CTC TCG TTA CCC GTC GGC CTC TTG TTG ATG TTC TGG TGC GGA GGG CAC
   E   S   N   G   Q   P   E   N   N   Y   K   T   T   P   P   V>

        1400          1410          1420          1430          1440
           *             *             *             *             *
  CTG GAC TCC GAC GGC TCC TTC TTC CTC TAC AGC AAG CTC ACC GTG GAC
  GAC CTG AGG CTG CCG AGG AAG AAG GAG ATG TCG TTC GAG TGG CAC CTG
   L   D   S   D   G   S   F   F   L   Y   S   K   L   T   V   D>

           1450          1460          1470          1480
              *             *             *             *
  AAG AGC AGG TGG CAG CAG GGG AAC GTC TTC TCA TGC TCC GTG ATG CAT
  TTC TCG TCC ACC GTC GTC CCC TTG CAG AAG AGT ACG AGG CAC TAC GTA
   K   S   R   W   Q   Q   G   N   V   F   S   C   S   V   M   H>

1490          1500          1510          1520          1530
   *             *             *             *             *
```

```
GAG GCT CTG CAC AAC CAC TAC ACG CAG AAG AGC CTC TCC CTG TCT CCG
CTC CGA GAC GTG TTG GTG ATG TGC GTC TTC TCG GAG AGG GAC AGA GGC
 E   A   L   H   N   H   Y   T   Q   K   S   L   S   L   S   P>

 1540          1550          1560          1570          1580
    *             *             *             *             *
GGT AAA CTG GAT CCC AAA CTC TGC TAC CTG CTG GAT GGA ATC CTC TTC
CCA TTT GAC CTA GGG TTT GAG ACG ATG GAC GAC CTA CCT TAG GAG AAG
 G   K   L   D   P   K   L   C   Y   L   L   D   G   I   L   F>

   1590          1600          1610          1620          1630
      *             *             *             *             *
ATC TAT GGT GTC ATT CTC ACT GCC TTG TTC CTG AGA GTG AAG TTC AGC
TAG ATA CCA CAG TAA GAG TGA CGG AAC AAG GAC TCT CAC TTC AAG TCG
 I   Y   G   V   I   L   T   A   L   F   L   R   V   K   F   S>

       1640          1650          1660          1670          1680
          *             *             *             *             *
AGG AGC GCA GAG CCC CCC GCG TAC CAG CAG GGC CAG AAC CAG CTC TAT
TCC TCG CGT CTC GGG GGG CGC ATG GTC GTC CCG GTC TTG GTC GAG ATA
 R   S   A   E   P   P   A   Y   Q   Q   G   Q   N   Q   L   Y>

          1690          1700          1710          1720
             *             *             *             *
AAC GAG CTC AAT CTA GGA CGA AGA GAG GAG TAC GAT GTT TTG GAC AAG
TTG CTC GAG TTA GAT CCT GCT TCT CTC CTC ATG CTA CAA AAC CTG TTC
 N   E   L   N   L   G   R   R   E   E   Y   D   V   L   D   K>

1730          1740          1750          1760          1770
   *             *             *             *             *
AGA CGT GGC CGG GAC CCT GAG ATG GGG GGA AAG CCG AGA AGG AAG AAC
TCT GCA CCG GCC CTG GGA CTC TAC CCC CCT TTC GGC TCT TCC TTC TTG
 R   R   G   R   D   P   E   M   G   G   K   P   R   R   K   N>

 1780          1790          1800          1810          1820
    *             *             *             *             *
CCT CAG GAA GGC CTG TAC AAT GAA CTG CAG AAA GAT AAG ATG GCG GAG
GGA GTC CTT CCG GAC ATG TTA CTT GAC GTC TTT CTA TTC TAC CGC CTC
 P   Q   E   G   L   Y   N   E   L   Q   K   D   K   M   A   E>

   1830          1840          1850          1860          1870
      *             *             *             *             *
GCC TAC AGT GAG ATT GGG ATG AAA GGC GAG CGC CGG AGG GGC AAG GGG
CGG ATG TCA CTC TAA CCC TAC TTT CCG CTC GCG GCC TCC CCG TTC CCC
 A   Y   S   E   I   G   M   K   G   E   R   R   G   K   G   G>

       1880          1890          1900          1910          1920
          *             *             *             *             *
CAC GAT GGC CTT TAC CAG GGT CTC AGT ACA GCC ACC AAG GAC ACC TAC
GTG CTA CCG GAA ATG GTC CCA GAG TCA TGT CGG TGG TTC CTG TGG ATG
 H   D   G   L   Y   Q   G   L   S   T   A   T   K   D   T   Y>

          1930          1940          1950
             *             *             *
GAC GCC CTT CAC ATG CAG GCC CTG CCC CCT CGC TAA
CTG CGG GAA GTG TAC GTC CGG GAC GGG GGA GCG ATT
 D   A   L   H   M   Q   A   L   P   P   P   *
```

FIGURE 8

SEQUENCE OF hCTMO1/G1/ZETA-CD28 FUSION RECOMBINANT CHIMERIC RECEPTOR

```
          10          20          30          40
          *           *           *           *
ATG TCT GTC CCC ACC CAA GTC CTC GGA CTC CTG CTG CTG TGG CTT ACA
TAC AGA CAG GGG TGG GTT CAG GAG CCT GAG GAC GAC GAC ACC GAA TGT
 M   S   V   P   T   Q   V   L   G   L   L   L   L   W   L   T>

50          60          70          80          90
*           *           *           *           *
GAT GCC AGA TGC GAT ATC CAG ATG ACT CAG AGT CCA AGT ACT CTC AGT
CTA CGG TCT ACG CTA TAG GTC TAC TGA GTC TCA GGT TCA TGA GAG TCA
 D   A   R   C   D   I   Q   M   T   Q   S   P   S   T   L   S>

  100         110         120         130         140
   *           *           *           *           *
GCC AGT GTA GGT GAT AGG GTC ACC ATC ACT TGT AGG AGT AGT AAA AGT
CGG TCA CAT CCA CTA TCC CAG TGG TAG TGA ACA TCC TCA TCA TTT TCA
 A   S   V   G   D   R   V   T   I   T   C   R   S   S   K   S>

    150         160         170         180         190
     *           *           *           *           *
CTC CTC CAT AGT AAC GGT GAC ACC TTC CTC TAT TGG TTC CAG CAG AAA
GAG GAG GTA TCA TTG CCA CTG TGG AAG GAG ATA ACC AAG GTC GTC TTT
 L   L   H   S   N   G   D   T   F   L   Y   W   F   Q   Q   K>

        200         210         220         230         240
         *           *           *           *           *
CCA GGT AAA GCC CCA AAG CTC CTC ATG TAT AGG ATG AGT AAC CTC GCC
GGT CCA TTT CGG GGT TTC GAG GAG TAC ATA TCC TAC TCA TTG GAG CGG
 P   G   K   A   P   K   L   L   M   Y   R   M   S   N   L   A>

          250         260         270         280
           *           *           *           *
AGT GGT GTA CCA TCT AGA TTC AGT GGT AGT GGT AGT GGT ACT GAG TTC
TCA CCA CAT GGT AGA TCT AAG TCA CCA TCA CCA TCA CCA TGA CTC AAG
 S   G   V   P   S   R   F   S   G   S   G   S   G   T   E   F>

290         300         310         320         330
 *           *           *           *           *
ACT CTC ACT ATC AGT AGT CTC CAG CCA GAT GAT TTC GCC ACT TAT TAT
TGA GAG TGA TAG TCA TCA GAG GTC GGT CTA CTA AAG CGG TGA ATA ATA
 T   L   T   I   S   S   L   Q   P   D   D   F   A   T   Y   Y>

  340         350         360         370         380
   *           *           *           *           *
TGT ATG CAG CAT CTC GAA TAT CCA TTC ACT TTC GGT CAG GGT ACT AAA
ACA TAC GTC GTA GAG CTT ATA GGT AAG TGA AAG CCA GTC CCA TGA TTT
 C   M   Q   H   L   E   Y   P   F   T   F   G   Q   G   T   K>

    390         400         410         420         430
     *           *           *           *           *
GTA GAA GTA AAA CGT ACG GGT GGC GGA GGG TCA GGT GGC GGA GGG TCA
CAT CTT CAT TTT GCA TGC CCA CCG CCT CCC AGT CCA CCG CCT CCC AGT
 V   E   V   K   R   T   G   G   G   G   S   G   G   G   G   S>

        440         450         460         470         480
         *           *           *           *           *
GGT GGC GGA GGG TCA GGT GGC GGA GGG TCA GGT GGC GGA GGG TCA CAG
CCA CCG CCT CCC AGT CCA CCG CCT CCC AGT CCA CCG CCT CCC AGT GTC
 G   G   G   G   S   G   G   G   G   S   G   G   G   G   S   Q>

          490         500         510         520
```

```
                *               *               *               *
ATT CAG CTG GTG CAG TCT GGA GCA GAG GTG AAG AAG CCT GGA TCT TCT
TAA GTC GAC CAC GTC AGA CCT CGT CTC CAC TTC TTC GGA CCT AGA AGA
 I   Q   L   V   Q   S   G   A   E   V   K   K   P   G   S   S>

530             540             550             560             570
  *               *               *               *               *
GTG AAG GTG TCT TGT AAG GCA TCT GGA TAC ACC TTC ACC GAC TAC TAC
CAC TTC CAC AGA ACA TTC CGT AGA CCT ATG TGG AAG TGG CTG ATG ATG
 V   K   V   S   C   K   A   S   G   Y   T   F   T   D   Y   Y>

   580             590             600             610             620
     *               *               *               *               *
ATT AAT TGG ATG AGA CAG GCA CCT GGA CAG GGA CTC GAG TGG ATT GGA
TAA TTA ACC TAC TCT GTC CGT GGA CCT GTC CCT GAG CTC ACC TAA CCT
 I   N   W   M   R   Q   A   P   G   Q   G   L   E   W   I   G>

       630             640             650             660             670
         *               *               *               *               *
TGG ATT GAC CCT GGA TCT GGA AAT ACA AAG TAC AAT GAG AAG TTC AAG
ACC TAA CTG GGA CCT AGA CCT TTA TGT TTC ATG TTA CTC TTC AAG TTC
 W   I   D   P   G   S   G   N   T   K   Y   N   E   K   F   K>

           680             690             700             710             720
             *               *               *               *               *
GGA AGA GCA ACA CTG ACA GTG GAC ACA TCC ACG AAT ACC GCC TAC ATG
CCT TCT CGT TGT GAC TGT CAC CTG TGT AGG TGC TTA TGG CGG ATG TAC
 G   R   A   T   L   T   V   D   T   S   T   N   T   A   Y   M>

               730             740             750             760
                 *               *               *               *
GAG CTG TCT TCT CTG AGA TCT GAG GAC ACA GCA TTC TAC TTC TGT GCA
CTC GAC AGA AGA GAC TCT AGA CTC CTG TGT CGT AAG ATG AAG ACA CGT
 E   L   S   S   L   R   S   E   D   T   A   F   Y   F   C   A>

770             780             790             800             810
  *               *               *               *               *
AGA GAG AAG ACC ACC TAC TAC TAC GCA ATG GAC TAC TGG GGA CAG GGA
TCT CTC TTC TGG TGG ATG ATG ATG CGT TAC CTG ATG ACC CCT GTC CCT
 R   E   K   T   T   Y   Y   Y   A   M   D   Y   W   G   Q   G>

   820             830             840             850             860
     *               *               *               *               *
ACA CTG GTG ACA GTG TCT TCT GCC TCA ACG AAG GGC CCG ACT AGT GAC
TGT GAC CAC TGT CAC AGA AGA CGG AGT TGC TTC CCG GGC TGA TCA CTG
 T   L   V   T   V   S   S   A   S   T   K   G   P   T   S   D>

       870             880             890             900             910
         *               *               *               *               *
AAA ACT CAC ACA TGC CCA CCG TGC CCA GCA CCT GAA CTC CTG GGG GGA
TTT TGA GTG TGT ACG GGT GGC ACG GGT CGT GGA CTT GAG GAC CCC CCT
 K   T   H   T   C   P   P   C   P   A   P   E   L   L   G   G>

           920             930             940             950             960
             *               *               *               *               *
CCG TCA GTC TTC CTC TTC CCC CCA AAA CCC AAG GAC ACC CTC ATG ATC
GGC AGT CAG AAG GAG AAG GGG GGT TTT GGG TTC CTG TGG GAG TAC TAG
 P   S   V   F   L   F   P   P   K   P   K   D   T   L   M   I>

               970             980             990            1000
                 *               *               *               *
TCC CGG ACC CCT GAG GTC ACA TGC GTG GTG GTG GAC GTG AGC CAC GAA
AGG GCC TGG GGA CTC CAG TGT ACG CAC CAC CAC CTG CAC TCG GTG CTT
 S   R   T   P   E   V   T   C   V   V   V   D   V   S   H   E>
```

```
1010          1020          1030          1040          1050
   *             *             *             *             *
  GAC CCT GAG GTC AAG TTC AAC TGG TAC GTG GAC GGC GTG GAG GTG CAT
  CTG GGA CTC CAG TTC AAG TTG ACC ATG CAC CTG CCG CAC CTC CAC GTA
    D   P   E   V   K   F   N   W   Y   V   D   G   V   E   V   H>

   1060          1070          1080          1090          1100
      *             *             *             *             *
  AAT GCC AAG ACA AAG CCG CGG GAG GAG CAG TAC AAC AGC ACG TAC CGT
  TTA CGG TTC TGT TTC GGC GCC CTC CTC GTC ATG TTG TCG TGC ATG GCA
    N   A   K   T   K   P   R   E   E   Q   Y   N   S   T   Y   R>

     1110          1120          1130          1140          1150
        *             *             *             *             *
  GTG GTC AGC GTC CTC ACC GTC CTG CAC CAG GAC TGG CTG AAT GGC AAG
  CAC CAG TCG CAG GAG TGG CAG GAC GTG GTC CTG ACC GAC TTA CCG TTC
    V   V   S   V   L   T   V   L   H   Q   D   W   L   N   G   K>

        1160          1170          1180          1190          1200
           *             *             *             *             *
  GAG TAC AAG TGC AAG GTC TCC AAC AAA GCC CTC CCA GCC CCC ATC GAG
  CTC ATG TTC ACG TTC CAG AGG TTG TTT CGG GAG GGT CGG GGG TAG CTC
    E   Y   K   C   K   V   S   N   K   A   L   P   A   P   I   E>

           1210          1220          1230          1240
              *             *             *             *
  AAA ACC ATC TCC AAA GCC AAA GGG CAG CCC CGA GAA CCA CAG GTG TAC
  TTT TGG TAG AGG TTT CGG TTT CCC GTC GGG GCT CTT GGT GTC CAC ATG
    K   T   I   S   K   A   K   G   Q   P   R   E   P   Q   V   Y>

1250          1260          1270          1280          1290
   *             *             *             *             *
  ACC CTG CCC CCA TCC CGG GAG GAG ATG ACC AAG AAC CAG GTC AGC CTG
  TGG GAC GGG GGT AGG GCC CTC CTC TAC TGG TTC TTG GTC CAG TCG GAC
    T   L   P   P   S   R   E   E   M   T   K   N   Q   V   S   L>

   1300          1310          1320          1330          1340
      *             *             *             *             *
  ACC TGC CTG GTC AAA GGC TTC TAT CCC AGC GAC ATC GCC GTG GAG TGG
  TGG ACG GAC CAG TTT CCG AAG ATA GGG TCG CTG TAG CGG CAC CTC ACC
    T   C   L   V   K   G   F   Y   P   S   D   I   A   V   E   W>

     1350          1360          1370          1380          1390
        *             *             *             *             *
  GAG AGC AAT GGG CAG CCG GAG AAC AAC TAC AAG ACC ACG CCT CCC GTG
  CTC TCG TTA CCC GTC GGC CTC TTG TTG ATG TTC TGG TGC GGA GGG CAC
    E   S   N   G   Q   P   E   N   N   Y   K   T   T   P   P   V>

        1400          1410          1420          1430          1440
           *             *             *             *             *
  CTG GAC TCC GAC GGC TCC TTC TTC CTC TAC AGC AAG CTC ACC GTG GAC
  GAC CTG AGG CTG CCG AGG AAG AAG GAG ATG TCG TTC GAG TGG CAC CTG
    L   D   S   D   G   S   F   F   L   Y   S   K   L   T   V   D>

           1450          1460          1470          1480
              *             *             *             *
  AAG AGC AGG TGG CAG CAG GGG AAC GTC TTC TCA TGC TCC GTG ATG CAT
  TTC TCG TCC ACC GTC GTC CCC TTG CAG AAG AGT ACG AGG CAC TAC GTA
    K   S   R   W   Q   Q   G   N   V   F   S   C   S   V   M   H>

1490          1500          1510          1520          1530
   *             *             *             *             *
  GAG GCT CTG CAC AAC CAC TAC ACG CAG AAG AGC CTC TCC CTG TCT CCG
  CTC CGA GAC GTG TTG GTG ATG TGC GTC TTC TCG GAG AGG GAC AGA GGC
```

```
  E   A   L   H   N   H   Y   T   Q   K   S   L   S   L   S   P>

 1540          1550          1560          1570          1580
    *             *             *             *             *
GGT AAA CTG GAT CCC AAA CTC TGC TAC CTG CTG GAT GGA ATC CTC TTC
CCA TTT GAC CTA GGG TTT GAG ACG ATG GAC GAC CTA CCT TAG GAG AAG
  G   K   L   D   P   K   L   C   Y   L   L   D   G   I   L   F>

    1590          1600          1610          1620          1630
       *             *             *             *             *
ATC TAT GGT GTC ATT CTC ACT GCC TTG TTC CTG AGA GTG AAG TTC AGC
TAG ATA CCA CAG TAA GAG TGA CGG AAC AAG GAC TCT CAC TTC AAG TCG
  I   Y   G   V   I   L   T   A   L   F   L   R   V   K   F   S>

        1640          1650          1660          1670          1680
           *             *             *             *             *
AGG AGC GCA GAG CCC CCC GCG TAC CAG CAG GGC CAG AAC CAG CTC TAT
TCC TCG CGT CTC GGG GGG CGC ATG GTC GTC CCG GTC TTG GTC GAG ATA
  R   S   A   E   P   P   A   Y   Q   Q   G   Q   N   Q   L   Y>

           1690          1700          1710          1720
              *             *             *             *
AAC GAG CTC AAT CTA GGA CGA AGA GAG GAG TAC GAT GTT TTG GAC AAG
TTG CTC GAG TTA GAT CCT GCT TCT CTC CTC ATG CTA CAA AAC CTG TTC
  N   E   L   N   L   G   R   R   E   E   Y   D   V   L   D   K>

1730          1740          1750          1760          1770
   *             *             *             *             *
AGA CGT GGC CGG GAC CCT GAG ATG GGG GGA AAG CCG AGA AGG AAG AAC
TCT GCA CCG GCC CTG GGA CTC TAC CCC CCT TTC GGC TCT TCC TTC TTG
  R   R   G   R   D   P   E   M   G   G   K   P   R   R   K   N>

 1780          1790          1800          1810          1820
    *             *             *             *             *
CCT CAG GAA GGC CTG TAC AAT GAA CTG CAG AAA GAT AAG ATG GCG GAG
GGA GTC CTT CCG GAC ATG TTA CTT GAC GTC TTT CTA TTC TAC CGC CTC
  P   Q   E   G   L   Y   N   E   L   Q   K   D   K   M   A   E>

    1830          1840          1850          1860          1370
       *             *             *             *             *
GCC TAC AGT GAG ATT GGG ATG AAA GGC GAG CGC CGG AGG GGC AAG GGG
CGG ATG TCA CTC TAA CCC TAC TTT CCG CTC GCG GCC TCC CCG TTC CCC
  A   Y   S   E   I   G   M   K   G   E   R   R   R   G   K   G>

        1880          1890          1900          1910          1920
           *             *             *             *             *
CAC GAT GGC CTT TAC CAG GGT CTC AGT ACA GCC ACC AAG GAC ACC TAC
GTG CTA CCG GAA ATG GTC CCA GAG TCA TGT CGG TGG TTC CTG TGG ATG
  H   D   G   L   Y   Q   G   L   S   T   A   T   K   D   T   Y>

           1930          1940          1950          1960
              *             *             *             *
GAC GCC CTT CAC ATG CAG GCC CTG CCC CCT CGC AGG AGT AAG AGG AGC
CTG CGG GAA GTG TAC GTC CGG GAC GGG GGA GCG TCC TCA TTC TCC TCG
  D   A   L   H   M   Q   A   L   P   P   R   R   S   K   R   S>

1970          1980          1990          2000          2010
   *             *             *             *             *
AGG CTC CTG CAC AGT GAC TAC ATG AAC ATG ACT CCC CGC CGC CCC GGG
TCC GAG GAC GTG TCA CTG ATG TAC TTG TAC TGA GGG GCG GCG GGG CCC
  R   L   L   H   S   D   Y   M   N   M   T   P   R   R   P   G>

 2020          2030          2040          2050          2060
    *             *             *             *             *
CCC ACC CGC AAG CAT TAC CAG CCC TAT GCC CCA CCA CGC GAC TTC GCA
```

```
GGG TGG GCG TTC GTA ATG GTC GGG ATA CGG GGT GGT GCG CTG AAG CGT
 P   T   R   K   H   Y   Q   P   Y   A   P   P   R   D   F   A>

   2070
     *
GCC TAT CGC TCC TGA
CGG ATA GCG AGG ACT
 A   Y   R   S   *
```

FIGURE 9

SEQUENCE OF hCTMO1 / h / CD28 RECOMBINANT CHIMERIC RECEPTOR

```
          10          20          30          40
           *           *           *           *
ATG TCT GTC CCC ACC CAA GTC CTC GGA CTC CTG CTG CTG TGG CTT ACA
TAC AGA CAG GGG TGG GTT CAG GAG CCT GAG GAC GAC GAC ACC GAA TGT
 M   S   V   P   T   Q   V   L   G   L   L   L   L   W   L   T>

50          60          70          80          90
 *           *           *           *           *
GAT GCC AGA TGC GAT ATC CAG ATG ACT CAG AGT CCA AGT ACT CTC AGT
CTA CGG TCT ACG CTA TAG GTC TAC TGA GTC TCA GGT TCA TGA GAG TCA
 D   A   R   C   D   I   Q   M   T   Q   S   P   S   T   L   S>

  100         110         120         130         140
   *           *           *           *           *
GCC AGT GTA GGT GAT AGG GTC ACC ATC ACT TGT AGG AGT AGT AAA AGT
CGG TCA CAT CCA CTA TCC CAG TGG TAG TGA ACA TCC TCA TCA TTT TCA
 A   S   V   G   D   R   V   T   I   T   C   R   S   S   K   S>

    150         160         170         180         190
     *           *           *           *           *
CTC CTC CAT AGT AAC GGT GAC ACC TTC CTC TAT TGG TTC CAG CAG AAA
GAG GAG GTA TCA TTG CCA CTG TGG AAG GAG ATA ACC AAG GTC GTC TTT
 L   L   H   S   N   G   D   T   F   L   Y   W   F   Q   Q   K>

       200         210         220         230         240
        *           *           *           *           *
CCA GGT AAA GCC CCA AAG CTC CTC ATG TAT AGG ATG AGT AAC CTC GCC
GGT CCA TTT CGG GGT TTC GAG GAG TAC ATA TCC TAC TCA TTG GAG CGG
 P   G   K   A   P   K   L   L   M   Y   R   M   S   N   L   A>

          250         260         270         280
           *           *           *           *
AGT GGT GTA CCA TCT AGA TTC AGT GGT AGT GGT AGT GGT ACT GAG TTC
TCA CCA CAT GGT AGA TCT AAG TCA CCA TCA CCA TCA CCA TGA CTC AAG
 S   G   V   P   S   R   F   S   G   S   G   S   G   T   E   F>

290         300         310         320         330
 *           *           *           *           *
ACT CTC ACT ATC AGT AGT CTC CAG CCA GAT GAT TTC GCC ACT TAT TAT
TGA GAG TGA TAG TCA TCA GAG GTC GGT CTA CTA AAG CGG TGA ATA ATA
 T   L   T   I   S   S   L   Q   P   D   D   F   A   T   Y   Y>

  340         350         360         370         380
   *           *           *           *           *
TGT ATG CAG CAT CTC GAA TAT CCA TTC ACT TTC GGT CAG GGT ACT AAA
ACA TAC GTC GTA GAG CTT ATA GGT AAG TGA AAG CCA GTC CCA TGA TTT
 C   M   Q   H   L   E   Y   P   F   T   F   G   Q   G   T   K>

    390         400         410         420         430
     *           *           *           *           *
GTA GAA GTA AAA CGT ACG GGT GGC GGA GGG TCA GGT GGC GGA GGG TCA
CAT CTT CAT TTT GCA TGC CCA CCG CCT CCC AGT CCA CCG CCT CCC AGT
 V   E   V   K   R   T   G   G   G   G   S   G   G   G   G   S>

       440         450         460         470         480
        *           *           *           *           *
GGT GGC GGA GGG TCA GGT GGC GGA GGG TCA GGT GGC GGA GGG TCA CAG
CCA CCG CCT CCC AGT CCA CCG CCT CCC AGT CCA CCG CCT CCC AGT GTC
 G   G   G   G   S   G   G   G   G   S   G   G   G   G   S   Q>
```

```
          490           500           510           520
           *             *             *             *
ATT CAG CTG GTG CAG TCT GGA GCA GAG GTG AAG AAG CCT GGA TCT TCT
TAA GTC GAC CAC GTC AGA CCT CGT CTC CAC TTC TTC GGA CCT AGA AGA
 I   Q   L   V   Q   S   G   A   E   V   K   K   P   G   S   S>

530           540           550           560           570
 *             *             *             *             *
GTG AAG GTG TCT TGT AAG GCA TCT GGA TAC ACC TTC ACC GAC TAC TAC
CAC TTC CAC AGA ACA TTC CGT AGA CCT ATG TGG AAG TGG CTG ATG ATG
 V   K   V   S   C   K   A   S   G   Y   T   F   T   D   Y   Y>

  580           590           600           610           620
   *             *             *             *             *
ATT AAT TGG ATG AGA CAG GCA CCT GGA CAG GGA CTC GAG TGG ATT GGA
TAA TTA ACC TAC TCT GTC CGT GGA CCT GTC CCT GAG CTC ACC TAA CCT
 I   N   W   M   R   Q   A   P   G   Q   G   L   E   W   I   G>

    630           640           650           660           670
     *             *             *             *             *
TGG ATT GAC CCT GGA TCT GGA AAT ACA AAG TAC AAT GAG AAG TTC AAG
ACC TAA CTG GGA CCT AGA CCT TTA TGT TTC ATG TTA CTC TTC AAG TTC
 W   I   D   P   G   S   G   N   T   K   Y   N   E   K   F   K>

        680           690           700           710           720
         *             *             *             *             *
GGA AGA GCA ACA CTG ACA GTG GAC ACA TCC ACG AAT ACC GCC TAC ATG
CCT TCT CGT TGT GAC TGT CAC CTG TGT AGG TGC TTA TGG CGG ATG TAC
 G   R   A   T   L   T   V   D   T   S   T   N   T   A   Y   M>

          730           740           750           760
           *             *             *             *
GAG CTG TCT TCT CTG AGA TCT GAG GAC ACA GCA TTC TAC TTC TGT GCA
CTC GAC AGA AGA GAC TCT AGA CTC CTG TGT CGT AAG ATG AAG ACA CGT
 E   L   S   S   L   R   S   E   D   T   A   F   Y   F   C   A>

770           780           790           800           810
 *             *             *             *             *
AGA GAG AAG ACC ACC TAC TAC TAC GCA ATG GAC TAC TGG GGA CAG GGA
TCT CTC TTC TGG TGG ATG ATG ATG CGT TAC CTG ATG ACC CCT GTC CCT
 R   E   K   T   T   Y   Y   Y   A   M   D   Y   W   G   Q   G>

  820           830           840           850           860
   *             *             *             *             *
ACA CTG GTG ACA GTG TCT TCT GCC TCA ACG AAG GGC CCG ACT AGT GAC
TGT GAC CAC TGT CAC AGA AGA CGG AGT TGC TTC CCG GGC TGA TCA CTG
 T   L   V   T   V   S   S   A   S   T   K   G   P   T   S   D>

    870           880           890           900           910
     *             *             *             *             *
AAA ACT CAC ACA TGC CCA CCG TGC CCA AAA GGG AAA CAC CTT TGT CCA
TTT TGA GTG TGT ACG GGT GGC ACG GGT TTT CCC TTT GTG GAA ACA GGT
 K   T   H   T   C   P   P   C   P   K   G   K   H   L   C   P>

        920           930           940           950           960
         *             *             *             *             *
AGT CCC CTA TTT CCC GGA CCT TCT AAG CCC TTT TGG GTG CTG GTG GTG
TCA GGG GAT AAA GGG CCT GGA AGA TTC GGG AAA ACC CAC GAC CAC CAC
 S   P   L   F   P   G   P   S   K   P   F   W   V   L   V   V>

          970           980           990          1000
           *             *             *             *
```

```
  GTT GGT GGA GTC CTG GCT TGC TAT AGC TTG CTA GTA ACA GTG GCC TTT
  CAA CCA CCT CAG GAC CGA ACG ATA TCG AAC GAT CAT TGT CAC CGG AAA
    V   G   G   V   L   A   C   Y   S   L   L   V   T   V   A   F>

1010          1020          1030          1040          1050
   *             *             *             *             *
  ATT ATT TTC TGG GTG AGG AGT AAG AGG AGC AGG CTC CTG CAC AGT GAC
  TAA TAA AAG ACC CAC TCC TCA TTC TCC TCG TCC GAG GAC GTG TCA CTG
    I   I   F   W   V   R   S   K   R   S   R   L   L   H   S   D>

   1060          1070          1080          1090          1100
      *             *             *             *             *
  TAC ATG AAC ATG ACT CCC CGC CGC CCC GGG CCC ACC CGC AAG CAT TAC
  ATG TAC TTG TAC TGA GGG GCG GCG GGG CCC GGG TGG GCG TTC GTA ATG
    Y   M   N   M   T   P   R   R   P   G   P   T   R   K   H   Y>

     1110          1120          1130          1140
        *             *             *             *
  CAG CCC TAT GCC CCA CCA CGC GAC TTC GCA GCC TAT CGC TCC TGA
  GTC GGG ATA CGG GGT GGT GCG CTG AAG CGT CGG ATA GCG AGG ACT
    Q   P   Y   A   P   P   R   D   F   A   A   Y   R   S   *
```

FIGURE 10

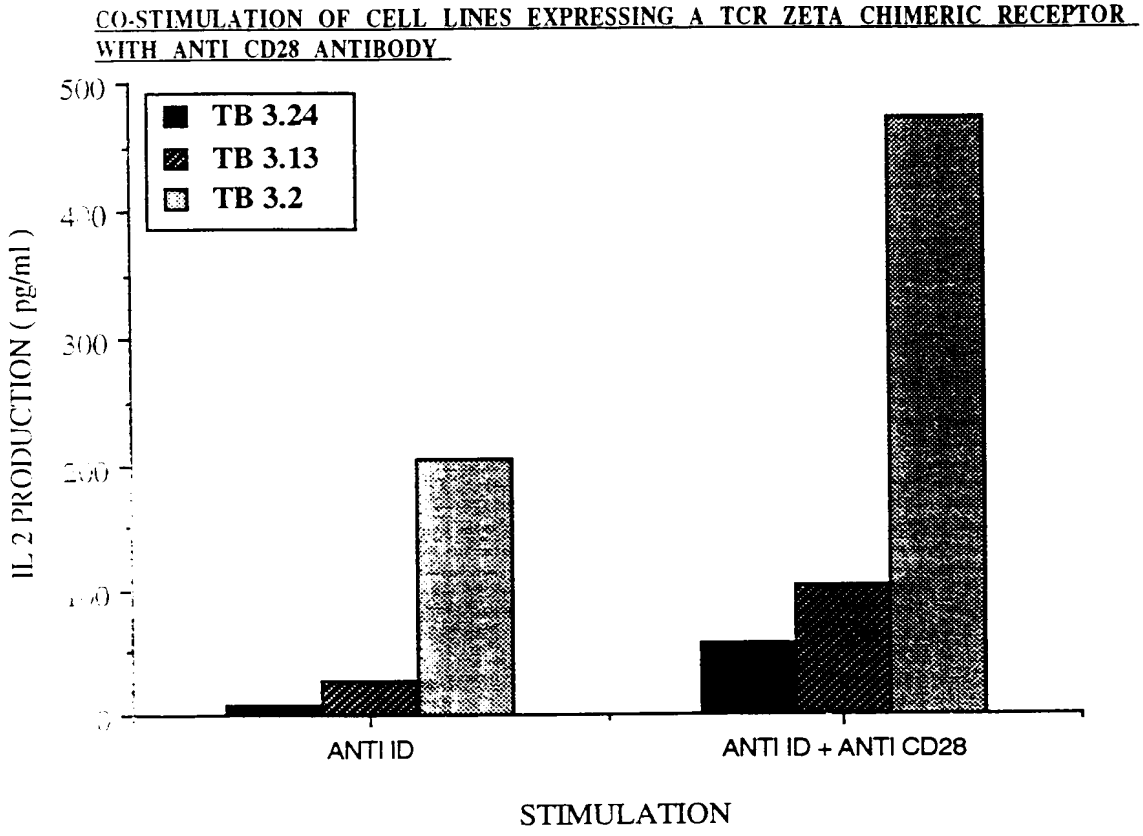

CO-STIMULATION OF CELL LINES EXPRESSING A TCR ZETA CHIMERIC RECEPTOR WITH ANTI CD28 ANTIBODY
TB 3.24
TB 3.13
TB 3.2
500
400
300
200
100
0
IL 2 PRODUCTION (pg/ml)
ANTI ID
ANTI ID + ANTI CD28
STIMULATION

FIGURE 11

STIMULATION WITH ANTIGEN POSITIVE CELLS,MCF-7

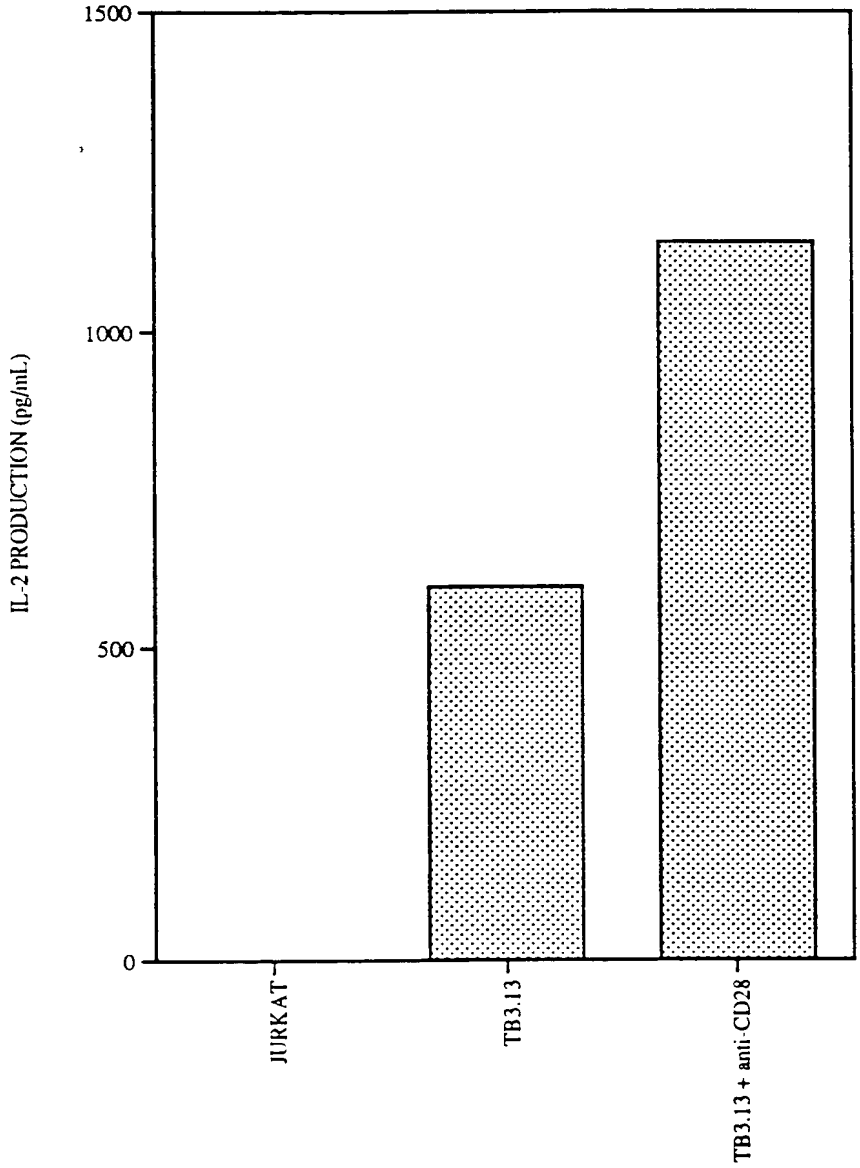
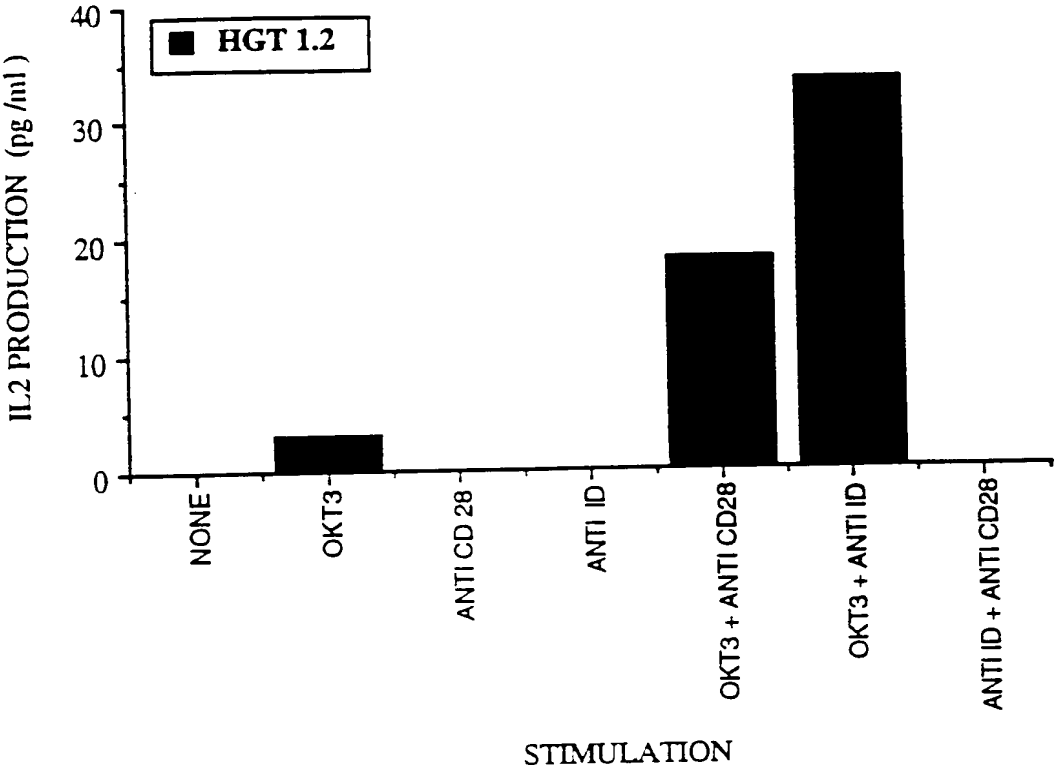


FIGURE 12

IL2 PRODUCTION IN RESPONSE TO VARIOUS STIMULI



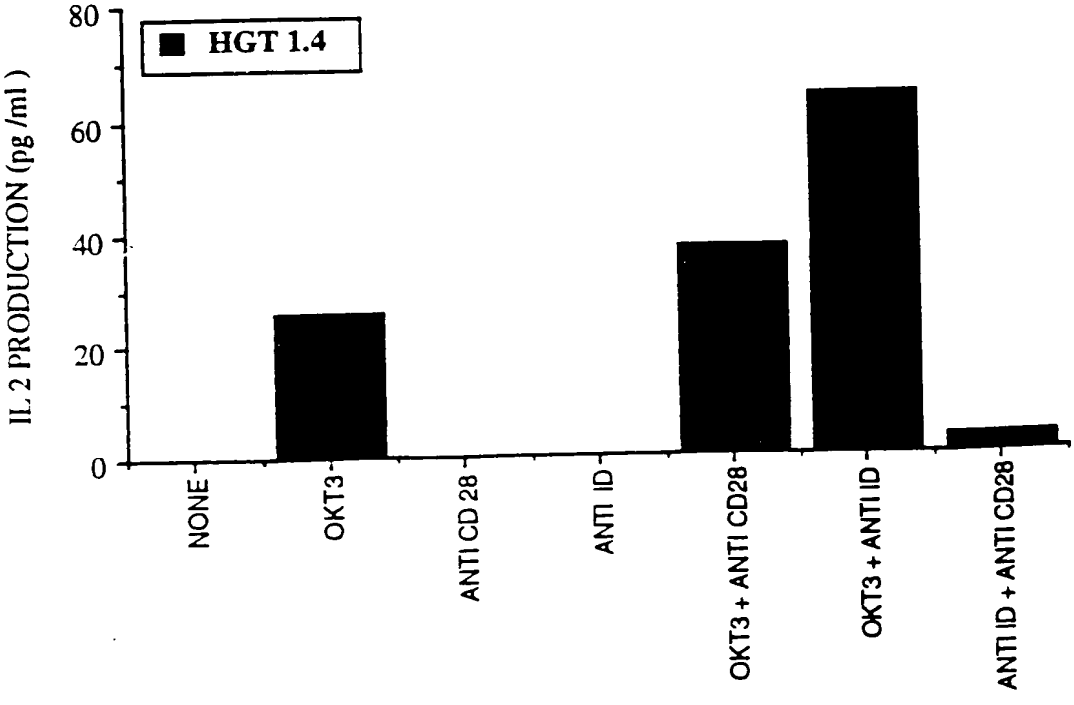


FIGURE 13

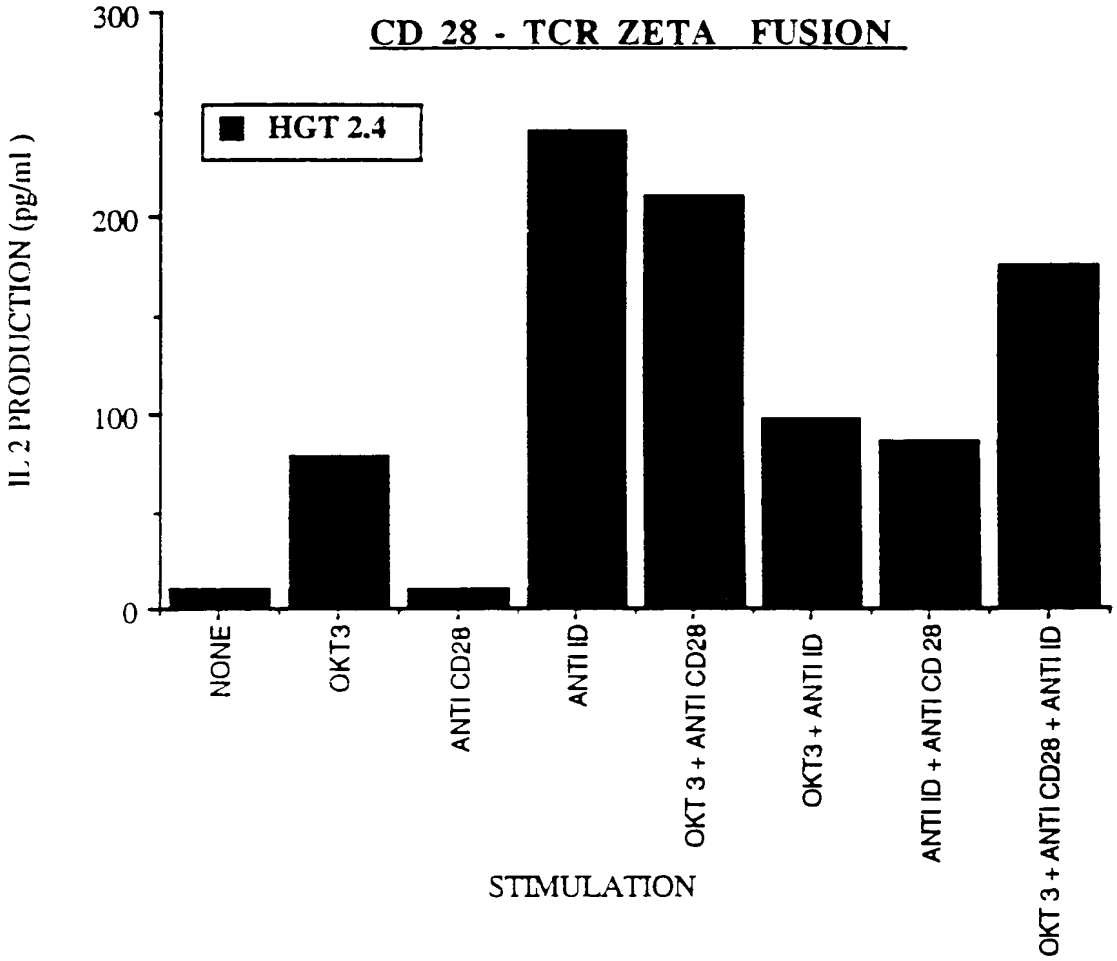
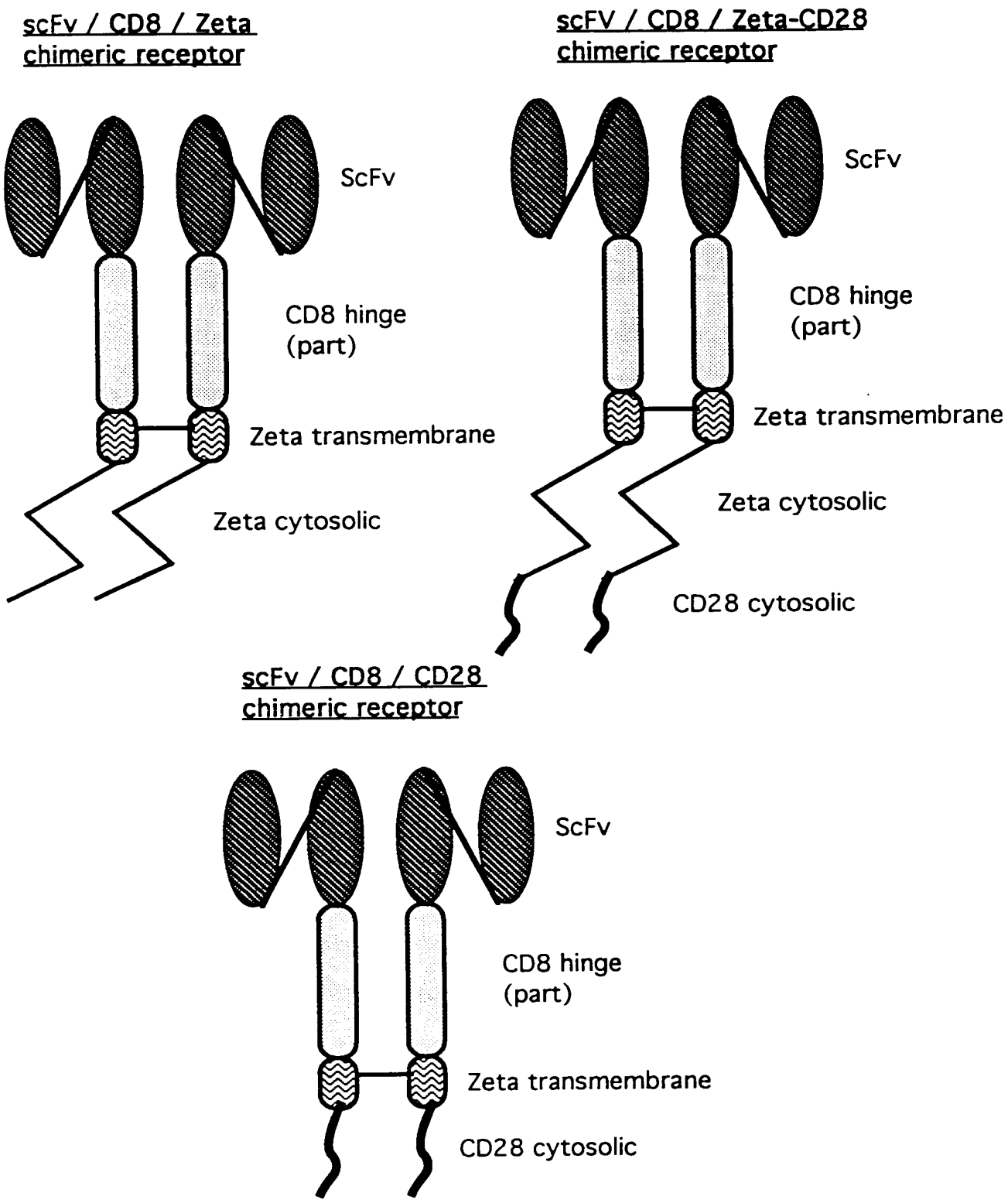

CD 28 - TCR ZETA FUSION
HGT 2.4
IL 2 PRODUCTION (pg/ml)
300
200
100
0
NONE
OKT3
ANTI CD28
ANTI ID
OKT 3 + ANTI CD28
OKT3 + ANTI ID
ANTI ID + ANTI CD 28
OKT 3 + ANTI CD28 + ANTI ID
STIMULATION

FIGURE 14


scFv / CD8 / Zeta chimeric receptor
ScFv
CD8 hinge (part)
Zeta transmembrane
Zeta cytosolic
scFV / CD8 / Zeta-CD28 chimeric receptor
ScFv
CD8 hinge (part)
Zeta transmembrane
Zeta cytosolic
CD28 cytosolic
scFv / CD8 / CD28 chimeric receptor
ScFv
CD8 hinge (part)
Zeta transmembrane
CD28 cytosolic

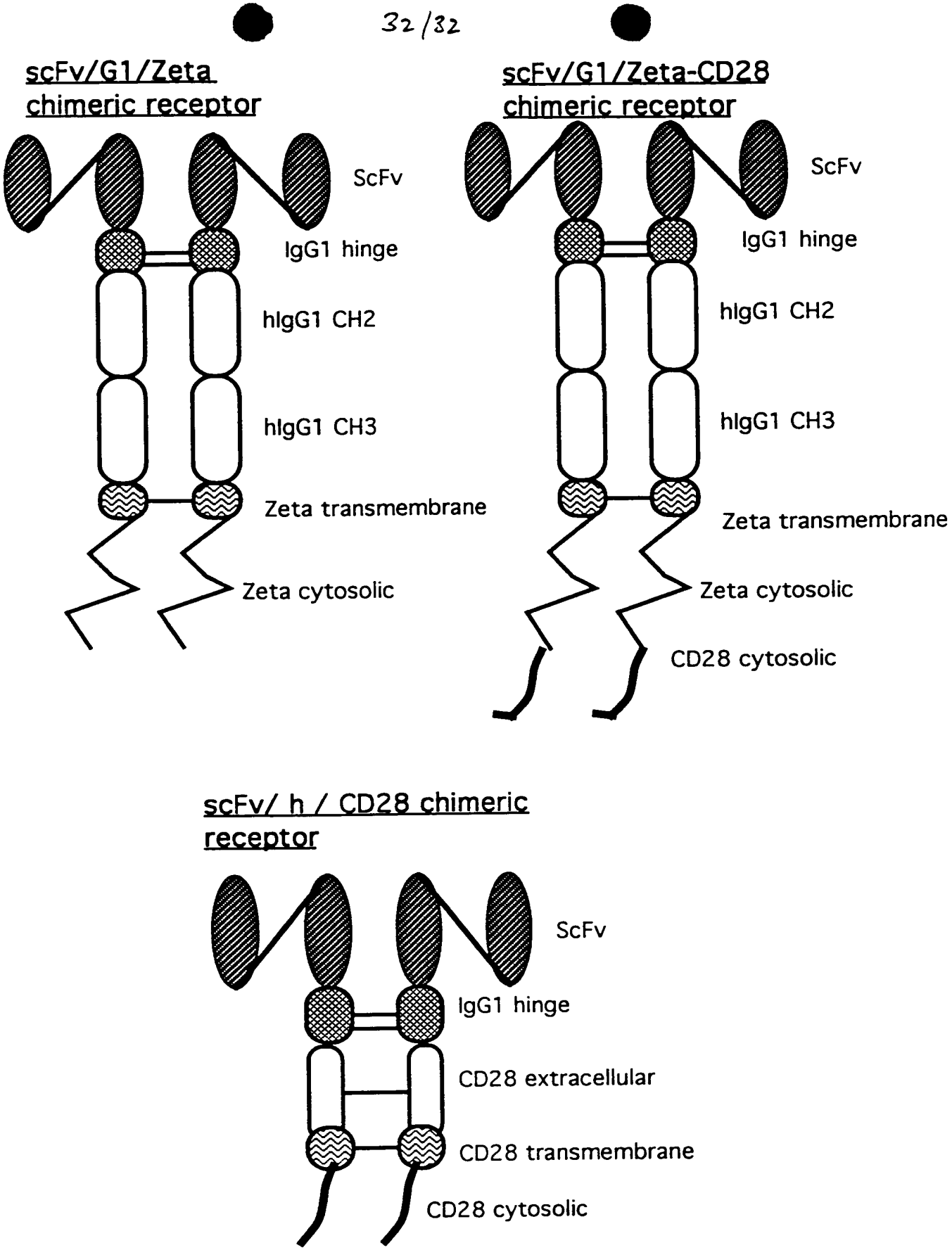

scFv/G1/Zeta chimeric receptor
ScFv
IgG1 hinge
hIgG1 CH2
hIgG1 CH3
Zeta transmembrane
Zeta cytosolic
scFv/G1/Zeta-CD28 chimeric receptor
ScFv
IgG1 hinge
hIgG1 CH2
hIgG1 CH3
Zeta transmembrane
Zeta cytosolic
CD28 cytosolic
scFv/ h / CD28 chimeric receptor
ScFv
IgG1 hinge
CD28 extracellular
CD28 transmembrane
CD28 cytosolic

FIGURE 15

PCT/GB 96 02109 23 12 96 - CARDMAT [redacted]

95 26121.9